



Habitual and acute exercise effects on salivary biomarkers in response to psychosocial stress



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ABSTRACT

Objective: Previous research suggests beneficial effects of physical exercise on stress reactivity due to cross-stressor adaptations of physiological stress response systems. However, results remain inconclusive and it is unclear whether only regular engagement in exercise modulates these physiological adaptations or if acute bouts of exercise can elicit similar adaptations. Thus, the aim of the current study was to investigate and compare the effects of habitual and acute exercise on physiological stress responses.

Methods: 84 male participants between 18 and 30 years (half of them were screened to be habitually high active or low active) were randomized into one of two groups: either an acute exercise intervention group ($n = 42$ with 50% being habitually high active) which engaged in 30 min of moderate-to-high intensity ergometer bicycling, or a control (placebo exercise) group which engaged in 30 min of light stretching ($n = 42$ with 50% being habitually high active). Following the intervention period, participants took part in a well validated psychosocial stress paradigm. Saliva samples were taken repeatedly to derive alpha-amylase and cortisol as stress-specific parameters. A multilevel growth curve approach was applied to analyse changes in the stress parameters over time.

Results: Both, acute and habitual exercise have shown to be positively related to stress reactivity. In particular, a reduction in stress activation was found for both types of exercise, but only habitual engagement in exercise exhibited a beneficial effect on peak cortisol levels.

Conclusions: Taken together, people can profit from regular exercise (i.e. reduced activity of stress-response systems). However, even acute bouts of exercise preceding stress exposure showed beneficial effects on stress reactivity. This finding is particularly important as it may provide a (self-)regulatory mechanism for people facing conceivable acute stress situations.

1. Introduction

Stress has many deleterious effects and is known to cause and exacerbate many physical and mental diseases (Chrousos, 2009). In search of factors counteracting these negative repercussions, research points towards a positive effect of regular engagement in physical exercise on stress-related complaints, including physical (e.g. heart disease, Gielen et al., 2015) and mental afflictions (e.g. anxiety, Rejeski et al., 1992, or well-being and affectivity, Wunsch et al., 2017) and has also provided evidence for the stress-buffering effect of physical

exercise (for reviews see Gerber and Pühse, 2009; Tsatsoulis and Fountoulakis, 2006). At first sight, exercise as a stress-buffering factor seems to be counterintuitive as it can also be seen as a stressor itself. When engaging in any kind of exercise, the two stress-response systems (i.e. the autonomic nervous system, ANS, and the hypothalamus pituitary adrenal, HPA, system), are activated in an intensity- and duration-dependent manner (Hackney, 2006). Though, the cross-stressor-adaptation (CSA) hypothesis postulates that regular engagement in physical exercise leads to unspecific adaptations (i.e. a reduced sensitivity) in stress-response systems, which can be transferred to subsequent

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homotypic (i.e. reduced stress responses to exercise and faster recovery) as well as to heterotypic (i.e. psychosocial) stressors (Sothmann et al., 1996). Whereas there is good evidence for advantages of regularly exercising (i.e. habitually active) individuals regarding homotypic stressors (e.g. Luger et al., 1987), evidence is diverse for heterotypic ones (Chauhan et al., 2015).

Stress reactivity can be operationalized by measuring outcomes of the two stress–response systems as a reaction to the stressor and can be divided into activation (comprising the reaction from stressor onset to peak reaction) and regulation (comprising the decline from peak reaction to baseline level). The vast majority of studies on effects of habitual exercise and/or fitness on heterotypic ANS-driven stress reactions focused on cardiovascular changes. In this respect, numerous reviews (e.g. Boutcher and Hamer, 2006; see Mücke et al., 2018, for a most recent review) and meta-analyses (e.g. Jackson and Dishman, 2006) exist, with only partial evidence for habitual exercise or fitness being associated with reduced stress reactivity. Recent trials using physiological ANS parameters as indicators for stress reactivity have reported similar inconsistent findings regarding autonomic responses with some studies observing increased reactivity and/or recovery (Jayasinghe et al., 2017), others diminished ones (Klaperski et al., 2014; Rimmele et al., 2007, 2009). To summarize results, Mücke et al. (2018) conducted a systematic review and included several physiological outcomes (e.g. heart rate, blood pressure, cardiac function), however revealing only limited support for the validity of the CSA hypothesis. High cardiovascular fitness levels were associated with a small, heterogeneous increase in stress reactivity. By analysing 9 studies on the effect of exercise or fitness on heart rate reactivity, the authors found only four studies to detect lower reactivity in more physically active or fitter participants and therefore a lower ANS reactivity to psychosocial stress. Three studies did not find any significant effects, and two studies revealed fitter participants to show an even higher heart rate reactivity than their less fit counterparts.

In recent years authors more often focused on endocrine parameters when examining the link between habitual exercise and/or fitness and stress. In this regard, saliva offers a useful tool to assess salivary cortisol (sCort) as an indicator of HPA axis activity (Foley and Kirschbaum, 2010). Based on assumptions of the CSA hypothesis, homotypic stressors cause a distinct rise of sCort concentration in response to the stressor (Hackney and Viru, 1999), whereas cross-stressor adaption effects of habitual exercise and/or fitness on heterotypic stressors are less clear. Undoubtedly, there are many studies examining effects of habitual exercise or fitness on stressor-induced ANS and HPA responses. However, they differ in many ways, like their operationalization of exercise (i.e. their definition of high/low exercise activity, or the kind of measurement thereof), their considered outcomes, the stress outcome measures used or age groups examined. Moreover, different stress induction methods, time of day when stress induction took place, and type of stressor may have influenced the effects, what may account for inconsistent results and makes studies difficult to compare. Sothmann (2006), for example, found no link between habitual exercise or fitness and endocrine HPA stress parameters in response to heterotypic stressors. In contrast, other studies (at least partly) support the CSA hypothesis for endocrine measures as they found a reduced sCort reactivity to a psychosocial stressor in trained compared to untrained men (Rimmele et al., 2009, 2007). However, habitual exercise showed no effect on stress recovery in these studies. Klaperski et al. (2014) confirmed these findings with reporting positive effects of a regular endurance exercise program and/or fitness for sCort reaction, but not for regulation. The systematic review conducted by Mücke et al. (2018) concluded that seven out of twelve studies supported the hypothesis of a reduced sCort reactivity in habitual exercising or fitter participants. Three of the reviewed studies found partial evidence for the CSA hypothesis, whereas two studies did not find any relationship of HPA activity and exercise or fitness. In conclusion, potential effects of habitual exercise or fitness on physiological and endocrine stress

reactivity still remain unclear.

Taken together, most studies focused on physiological measures like heart rate or blood pressure to examine ANS driven responses and on sCort to examine HPA driven responses when examining the CSA hypothesis. Until today, research on salivary alpha-amylase (sAA) as a biomarker for ANS activity as well as on sCort as an endocrine indicator for HPA axis activity is slowly emerging. According to the systematic review of Mücke et al. (2018), HPA (sCort) responses were diminished in habitually high active (i.e. habitually exercising) and fitter individuals in about 60% of the studies. This CSA effect was only found in about 40% of the studies measuring ANS responses (i.e. heart rate). Of note, these different stress markers show only weak correlations under acute stress (e.g. Engert et al., 2011; Thoma et al., 2012). Missing correlations, however, are not surprising as sCort and cardiovascular measures as well as sAA represent different aspects of the bodily defence system.

Studies mentioned above focused on habitual exercise or fitness to test the CSA-hypothesis. However, as acute (i.e. a single bout of) exercise is known to buffer stress-related complaints like self-reported distress and anxiety (Rejeski et al., 1992), the question about potential stress-buffering and CSA effects of a single bout of exercise on heterotypic stress reactivity emerged in recent years. Summarizing related knowledge, Taylor (2000) found only four out of 14 studies to not elicit any positive effect of acute exercise on stress reactivity. More recent reviews (Boutcher and Hamer, 2006; Hamer et al., 2006) confirmed these findings and proposed a reduced physiological stress reactivity of the ANS (regarding heart rate and blood pressure) in most of their reviewed studies. Furthermore, Alderman et al. (2007) investigated the effects of different exercise intensities and time of exposure to a psychological stressor (arithmetic task) on heart rate and blood pressure. Both low (50–55% of VO₂max) and high intensity exercise (75–80% of VO₂max) attenuated blood pressure and heart rate reactivity at 5, 30 and 60 min following exercise compared to a control condition. Moreover, participants of the high intensity exercise group recovered fastest in all three conditions regarding heart rate (Alderman et al., 2007). Until today, the acute stress-buffering effect of exercise on HPA responses has only been investigated twice (Wood et al., 2018; Zschucke et al., 2015). The study conducted by Zschucke et al. (2015) showed for a first time that also a single bout of exercise significantly reduced sCort reaction. Wood et al. (2018) revealed that fitter individuals had reduced sCort secretion during psychosocial stress after a submaximal walk and therefore confirmed the CSA hypothesis for HPA responses. However, nothing is known about stress regulation after cessation of the stressor.

Taken together, most studies examined either habitual or acute exercise and merely used sCort as a salivary biomarker for HPA axis response. ANS axis response is commonly represented by measures of heart rate, heart rate variability or blood pressure, not using salivary biomarkers. Authors did usually not distinguish between reaction to and recovery from a stressor when reporting results on stress reactivity, even if inconclusive findings were obtained. To extend this line of research, the present study aimed to investigate the effects of habitual and acute exercise at a time on salivary ANS and HPA biomarkers preceding, during and following acute psychosocial stress exposure to account for stress reaction and recovery in male adults. Based on previous studies it was assumed that participants being habitually or acutely active (i.e. exercising) show reduced stress reaction and faster recovery for both, sCort and sAA derived as salivary biomarkers for HPA and ANS axis activity.

2. Methods

2.1. Participants

Participants were recruited via announcements at the University and in local newspapers, in social networks as well as by direct

communication. Focus of the current study was on male subjects because hormonal changes during the menstrual cycle or taking contraceptives are expected to cause further variation in stress responses and controlling for this would not have been feasible. Age range was restricted to draw comparisons to previous research on the CSA and to exclude the impact of age on salivary stress markers (Strahler et al., 2010; van Cauter et al., 1996). Moreover, since many factors are known to influence sCort and sAA reactivity (Nater et al., 2007), eligibility criteria were defined. Accordingly, participants were excluded if they were younger than 18/older than 30 years, engaged in shift work (i.e. regularly change working hours from day to night), were studying Psychology or had prior psychological experiment attendance, had a body-mass-index lower than 19/higher than 30, were smoking or reported regular alcohol consumption based on the alcohol use disorders identification test (Babor et al., 2001), had any regular medication intake, had any exercise contradictions or suffered from any acute (e.g. flu) or chronic (e.g. heart insufficiency/disease, allergies) medical or psychological illness. Additionally, eligible participants had to meet requirements regarding their amount of regular (i.e. habitual) exercise, as they were only eligible if they either reported no regular exercise (neither aerobic nor anaerobic) for the past six months (in the following labelled as “inactive”) or if they engaged in aerobic exercise for at least 150 min per week during the same period¹ (in the following labelled as “active”). The final sample consisted of 84 male adults ($M_{\text{age}} = 24.00$ years, $SD = 3.17$). Detailed participant flow is presented in Fig. 1.

2.2. Procedure

The whole procedure was approved by the Ethics Committee of the University of Freiburg (approval number 206/16) and followed principles of the Declaration of Helsinki. As depicted in Fig. 2, participants completed an online pre-screening to ensure them meeting inclusion criteria. Afterwards, eligible participants were invited to the laboratory twice. Within the first laboratory session, participants completed computer-based questionnaires consisting of anamnestic as well as demographic information and psychological measures (i.e. their chronic stress level and their trait anxiety). Additionally, physical fitness was objectively assessed by means of a bicycle ergometer procedure (WHO procedure, Trappe and Löllgen, 2000) in order to (1) confirm participants' self-reports about their habitual exercise activity (as half of them were screened to be endurance athletes and therefore were expected to show comparably high fitness levels as opposed to their inactive counterparts) and to (2) examine individual maximal load levels. During the course of the fitness assessment, participants were asked to rate their perceived physical exhaustion based on a Borg rating scale ranging from 6 (very little) to 20 (very high) (Borg, 2004) with every load increment. The whole session lasted between 60 and 75 min.

One week following this examination, participants were invited to the laboratory a second time for the stress–response-assessment. This session was scheduled between 11 am and 3 pm in order to minimize the effect of circadian variations in salivary biomarkers (e.g. Nater et al., 2007). Regarding stress–response assessment, participants were instructed to refrain from any strenuous physical activity 3 days prior to examination and to refrain from any activity (including fast walking or cycling for example), from alcohol, coffee, caffeinated liquids, black or

green tea and to not take any medication (like acetylsalicylic acid, for example) for 24 h prior to the testing session. Participants were also requested to refrain from sugar (especially liquids or chewing gum) and not to eat or brush their teeth at least 2 h prior to testing. All participants were asked about adherence and confirmed they followed these instructions.

Preceding the examination, all participants (active vs. inactive) were randomized into one of two experimental groups (intervention vs. control) and attended the following procedure in groups of three. Participants allocated to the intervention group ($n = 42$ with 50% active) engaged in an acute exercise session with ergometer bicycling at 70% of their individual maximum load in Watts/kg examined at the first laboratory session, whereas participants of the control group ($n = 42$ with 50% active) engaged in 30 min of light stretching (placebo exercise), not including any aerobic component. The placebo exercise was conducted based on previously published protocols (Knubben et al., 2007; Zschucke et al., 2015). Following this 30-min period of either acute or placebo exercise, stress response assessment started. Here, the Trier Social Stress Test for Groups (TSST-G; von Dawans et al., 2011) was used as a well-validated procedure for stress induction, which has shown to be the best stress evoking paradigm (Dickerson and Kemeny, 2004). The TSST-G is known to elicit two- to threefold increases in ANS and HPA responses and is comprised of a free speech and a mental arithmetic task. For stress reactivity assessment, cortisol release is a valid indicator for HPA activity in response to an acute psychosocial stressor, especially when psychosocial stress is induced by tasks containing socio-evaluative threat and uncontrollability (Dickerson and Kemeny, 2004). The free, biologically active cortisol fraction in the blood can be reliably and validly assessed through the measurement of free cortisol in saliva. sCort is known to gradually increase within 10 min in resonance to a stressor, reaching its peak 10 to 30 min after stressor cessation (Foley and Kirschbaum, 2010). On the other hand, sAA has been shown to be a sensitive biomarker for stress-related changes which reflect the sympathetic activity of the ANS system (Nater and Rohleder, 2009), representing an immediate reaction to a stressor without delay. Following the TSST-G, participants stayed in the laboratory for another 60 min to ensure full recovery of biological parameters.

3. Measures

3.1. Biological measures

sCort and sAA were used as indicators for HPA and ANS response patterns, respectively. After participants arrived for stress–response-assessment they were asked to rinse their mouth with tap water to eliminate any confounding particles in saliva samples. Saliva samples were collected from each participant using a commercially available sampling device with polyester swabs (Salivettes[®]; Sarstedt, Nümbrecht, Germany) and were collected at seven time points during the course of the second assessment session: The first sample was taken 10 min after arrival of participants at the laboratory (T1), followed by a second sample immediately after the intervention (T2) and two additional samples right before (T3) and right after the TSST-G (T4). The remaining samples were taken 10 min (T5), 30 min (T6), and 65 min (T7) after stressor cessation (see Fig. 2 for an overview of sampling occasions). Afterwards, participants were thanked, debriefed and received feedback about their current fitness level. The whole testing session lasted about 3.5 to 4 h. Subsequently following sampling, Salivettes were stored at -20°C and were sent to Dresden Lab-Service GmbH (Germany) after study completion for biochemical analysis, where they were thawed and spun at 4°C 3000 rpm for 3 min to obtain clear saliva. Free cortisol concentrations (nmol/l) were determined by a luminescence immunoassay for the in vitro diagnostic quantitative determination of cortisol in human saliva (IBL International). Samples were immediately re-frozen after determination and were sent to the

¹ The period of six months was chosen as the transtheoretical model assumes that people being in the “maintenance stage” (what defines a habit) need to keep track on their regular exercise activity for at least six months to maintain this habit. The duration of 150 min per week was chosen to ensure participants achieved or even exceeded the recommendations of the World Health Organization to elicit beneficial health protecting effects (World Health Organization, 2010). Participants' self-reported amount of exercise was validated in the first laboratory session (fitness assessment). Here, correlation between habitual exercise activity and fitness was highly significant ($r = .637$; $p < .001$).

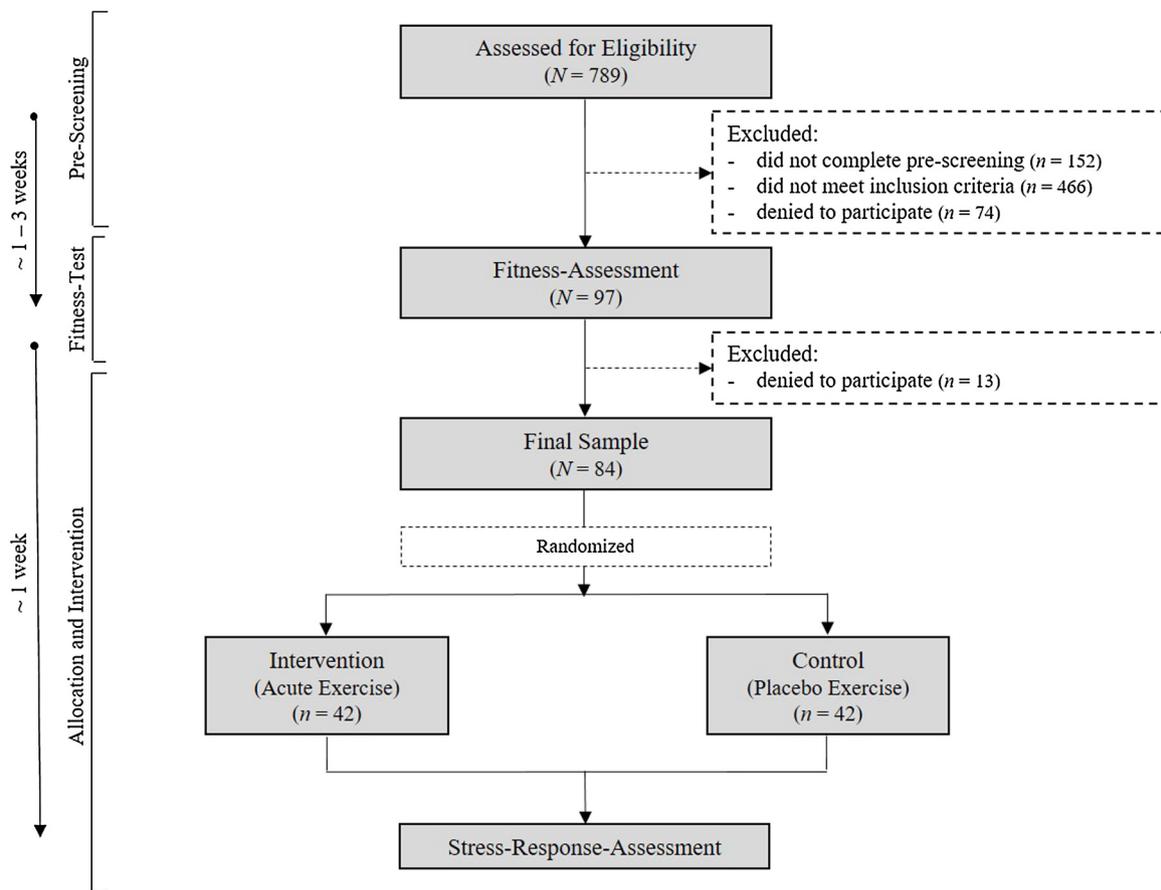


Fig. 1. Participant flow diagram.

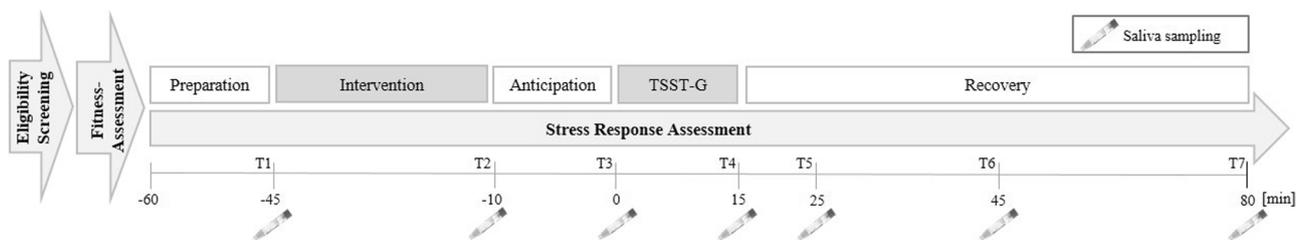


Fig. 2. Study design: sequence of events and measurements on timeline.

biochemical laboratory of the Department of Clinical Biopsychology in Marburg. After thawing and re-centrifuging, sAA activity was measured using a kinetic colorimetric test and reagents obtained from Roche (Roche Diagnostics, Mannheim, Germany). In short, saliva was diluted 1:625 using 0.9% saline solution. The reagents contained the enzyme alpha-amylase in a specified amount and alpha glucosidase, which converts the substrate ethylenedinitrophenyl to p-nitrophenol. The rate of formation of p-nitrophenol is directly proportional to the samples' amylase activity and was detected using an absorbance reader at 405 nm (Spectrostar nano, BMG Labtech, Ortenberg, Germany). Inter- and intra-assay coefficients of variation were below 8.5% for both determinations.

3.2. Habitual exercise

Habitual exercise was assessed using the validated German Physical Activity, Exercise and Sport Questionnaire (BSA-F, Fuchs et al., 2015). Subjects were asked to name sport and exercise activities they regularly engaged in within the last four weeks and to indicate frequency and duration of each episode in minutes. For each participant, an exercise

activity amount was calculated in minutes per week by multiplying frequency by duration and dividing it by four, as subjects were asked to indicate monthly frequency. All amounts were then added up and divided by 60 to obtain a total exercise activity index in hours per week.

3.3. Chronic stress and trait anxiety

To control for a possible impact of chronic stress exposure and trait-anxiety on HPA and ANS reactivity, participants completed the Screening Scale of Chronic Stress (SSCS; short form of the Trier Inventar zum chronischen Stress (TIGS), Schulz et al., 2004) as well as the trait part of the State-Trait-Anxiety Inventory (STAI-T; Spielberger and Gorsuch, 2010). Internal consistency was good for all scales, with Cronbach's $\alpha = .89$ for SSCS, and $\alpha = .93$ for STAI-T.

3.4. Data analysis

3.4.1. Data preparation

Both, sCort and sAA values exhibited notable deviations from normal distribution. Therefore, sAA data were log-transformed to

Table 1
Means and standard deviations for subject characteristics divided by experimental group.

	Intervention group (acute exercise) (n = 42)	Control group (placebo exercise) (n = 42)	t(82)	p
Age (years)	24.17 (3.07)	23.83 (2.32)	0.48	.632
BMI (kg/m ²)	22.70 (2.86)	22.09 (2.33)	1.07	.288
Habitual Exercise (h/week)	3.88 (4.79)	4.44 (5.99)	-0.47	.640
Fitness (watt/kg)	4.00 (0.82)	4.07 (0.93)	-0.34	.735
Trait Anxiety	37.38 (11.19)	36.76 (9.22)	0.28	.780
Chronic Stress	15.62 (7.62)	16.19 (7.27)	-0.35	.727

reduce skewness. For sCort, however, a Box-Cox power transformation was applied as specified by Miller and Plessow (2013). More specifically, data was transformed using the formula $X' = (X^{0.26} - 1)/0.26$. This was preferred over the more traditional log-transformation of cortisol data because it has been shown to produce superior results (Miller and Plessow, 2013).

Since the aerobic exercise intervention preceding the stress-response-assessment is also expected to have great influence on the considered parameters (Gerber and Pühse, 2009), the sample taken at T3 is defined as a baseline for stress-response-assessment. Inspection of the sCort and sAA progression plots suggested that participants had fully recovered (i.e. values returned to baseline) at T7 for sCort and at T5 for sAA. Paired t-Tests confirmed this impression as the sCort sample at T7 ($t(82) = 0.85, p = .398, d = 0.09$) and the sAA sample at T5 ($t(81) = 0.59, p = .558, d = 0.04$) did not differ from their respective baseline measurement. Accordingly, in sAA data, the two final samples (T6 and T7) were omitted for further analyses.

3.4.2. Statistical analyses

A multilevel growth curve approach using HLM 7.0 (Raudenbush et al., 2011) was applied to analyse changes in the two salivary biomarkers over time, as this approach allows for concurrent estimation of both, within-subject trajectories on level 1 and interindividual differences on level 2 (Singer and Willett, 2003). Initial analyses revealed differences in the temporal dynamics of sCort stress responses between groups. More specifically, participants of the control (placebo exercise) group ($M = 24.76, SD = 9.69$) peaked on average 5 min later than participants of the intervention (aerobic exercise) group ($M = 19.62, SD = 11.70$). This difference was statistically significant ($F(1, 82) = 4.82, p < .05, \eta^2 = .06$). Since peak latency variability can impact the assessment and interpretation of stress reaction and/or

regulation, these differences were adjusted using two-piece multilevel growth curve models with landmark registration (GCM-LR; Lopez-Duran et al., 2014). Within this approach, each subjects' curve is anchored to its individual sCort peak. There were no differences in peak latency between groups for sAA ($F(1, 81) = 0.59, p = .445, \eta^2 = .01$). Accordingly, each participant's curve was anchored to group peak for sAA (i.e. directly following stress induction at T4).

For both dependent variables, two time variables were considered to model participants' individual stress curve on level 1. The first variable comprised time points prior to peak and therefore modelled stress reaction (activation), whereas the second variable represented the time points after peak and therefore represented participants' ability to recover from the stressor (regulation). Both time variables were added simultaneously to build an unconditional growth model comprising random intercept and random slopes to predict sCort or sAA level:

$$Y_{ij} = \beta_{0j} + \beta_{1j} \cdot \text{activation}_{ij} + \beta_{2j} \cdot \text{regulation}_{ij} + e_{ij}$$

In a subsequent step, a conditional growth model was created by adding variables to level 2 in order to simultaneously examine the impact of habitual and acute exercise as well as the interaction between them on peak levels (intercept), the activation and the regulation slope:

$$\beta_{0j} = \gamma_{00} + \gamma_{01} \cdot \text{habitual}_j + \gamma_{02} \cdot \text{acute}_j + \gamma_{03} \cdot \text{acute}_j \cdot \text{habitual}_j + \nu_{0j}$$

$$\beta_{1j} = \gamma_{10} + \gamma_{11} \cdot \text{habitual}_j + \gamma_{12} \cdot \text{acute}_j + \gamma_{13} \cdot \text{acute}_j \cdot \text{habitual}_j + \nu_{1j}$$

$$\beta_{2j} = \gamma_{20} + \gamma_{21} \cdot \text{habitual}_j + \gamma_{22} \cdot \text{acute}_j + \gamma_{23} \cdot \text{acute}_j \cdot \text{habitual}_j + \nu_{2j}$$

Here, acute exercise was dummy-coded representing participants' assignment to one of the experimental groups (0 = placebo exercise, 1 = acute exercise), whereas habitual exercise was added as an interval-scaled variable representing total exercise amount in hours per week as indicated in the BSA-F questionnaire.

4. Results

4.1. Preliminary analyses

Table 1 presents participants' study characteristics demonstrating that randomization of subjects to the experimental groups worked efficiently, as participants from the different groups are comparable in all descriptive variables considered in this examination.

For descriptive purposes, raw data for sCort and sAA are presented

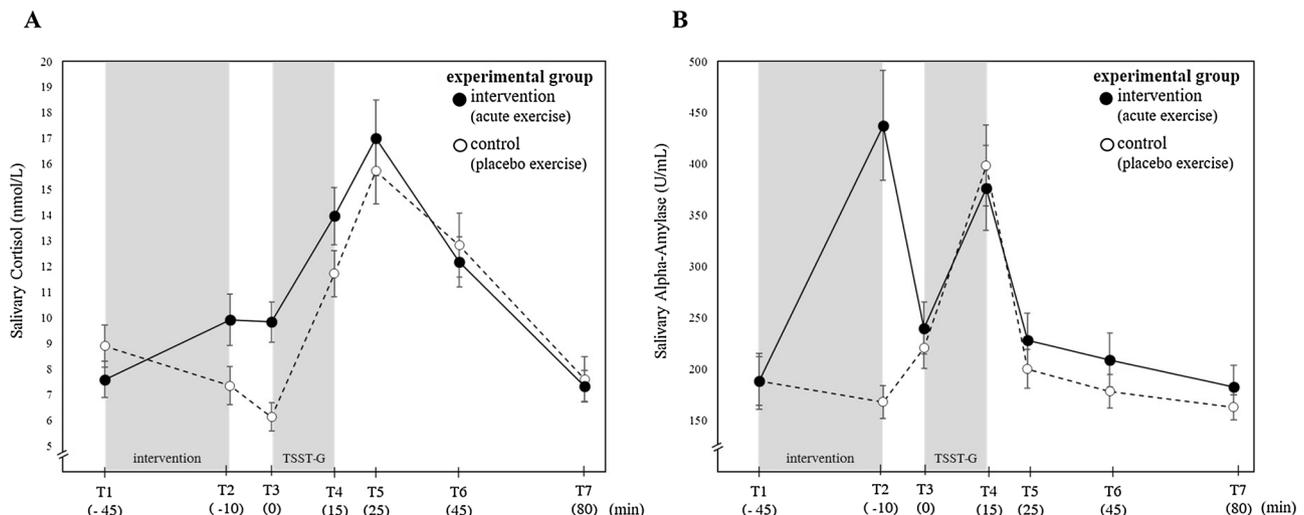


Fig. 3. Salivary Cortisol (A) and salivary alpha-Amylase (B) separated by experimental group.

in Fig. 3.

First, a manipulation check² was conducted to control for a successful stress induction. Although it has not been finally clarified yet, an increase of 10% in sCort and sAA is considered a significant stress response (Katz and Peckins, 2017). In the present study, 83% of participants exceeded this cut-off for sCort and 88% for sAA. The mean increase was 162% over baseline measurement for sCort and 103% for sAA. Overall, it can be concluded that stress induction was successful.

4.2. Two-piece multilevel growth curve models

Table 2 contains the results for both, unconditional and conditional growth model, for sCort and sAA trajectories.

The coefficients of the unconditional growth model revealed that change in trajectories over time is as expected. This is demonstrated by an increase in sCort and sAA values within the activation sequence, indicated by significant positive regression weights (i.e., $coef = 0.059$ for sCort and $coef = 0.016$ for sAA), and a deceleration after the peak, represented by significant negative regression weights (i.e., $coef = -0.030$ for sCort and $coef = -0.026$ for sAA). Subsequently, detailed results from the conditional model regarding peak levels, activation and regulation for both parameters will be described. Additionally, Fig. 4 displays fitted trajectories for four prototypical individuals to visually inspect results of the conditional model. To ease interpretation of Fig. 4 sCort and sAA values are retransformed to the original metric.

4.2.1. Peak level

There was no significant effect of habitual and acute exercise on sAA peak level. However, habitual exercise had a significant effect on peak sCort ($coef = -0.092$, $p < .001$), showing that for every hour per week that a participant exercised, transformed sCort peak concentrations reduce by 0.092 in reaction to the TSST-G. Additionally, a trend-level effect could be detected for the interaction between habitual and acute exercise on peak sCort ($coef = -0.072$, $p = .076$).

To generate an understanding of this effect, separate analyses for the experimental groups were performed. Conditional growth models indicated that the beneficial effect of habitual exercise on peak sCort concentrations was only salient for participants in the control group ($coef = -0.092$, $p < .001$), but not for participants allocated to the intervention group ($coef = -0.021$, $p = .505$).

4.2.2. Activation

Results for the conditional model revealed a significant effect for both, acute ($coef = -0.031$, $p < .05$) and habitual exercise ($coef = -0.002$, $p < .05$) on sCort reaction due to stress exposure, indicating decreased activation slopes for both types of exercise. For sAA, a similar pattern emerged. However, only the effect for acute exercise reached significance ($coef = -0.009$, $p < .05$), whereas habitual exercise only affected sAA reaction by tendency ($coef = -0.001$, $p = .098$).

4.2.3. Regulation

For sCort regulation, a significant effect of habitual exercise was detected ($coef = 0.001$, $p < .001$) as well as a trend-level effect for acute exercise ($coef = 0.001$, $p = .085$). Both effects suggest diminished effects of exercise on sCort regulation (i.e. recovery). sAA recovery evoked similar results, although only the regression weight associated with acute exercise reached significance ($coef = 0.012$, $p < .05$).

4.2.4. Control variables

To test the robustness of the present results, it was examined

whether the coefficients of the conditional model changed after controlling for other variables including trait anxiety (STAI-T; Spielberger and Gorsuch, 2010), chronic stress (SSCS; Schulz et al., 2004), BMI (grand-centered), and test time. Regarding sCort, none of these variables had a significant effect on either peak values or slopes. Additionally, all other results remained significant and in the same direction. Regarding sAA, BMI and test time had a significant effect on pre-peak slopes with individuals who showed an above-average BMI and were tested at a later time point exhibit higher slope estimates. However, all other results remained the same, especially the effects of habitual activity and the intervention. Accordingly, it can be assumed that the results from the conditional model are fairly robust.

5. Discussion

The current study aimed to investigate the concurrent effects of habitual and acute exercise on salivary stress parameters. To represent both, HPA and ANS axis response, sCort and sAA were considered as indicators of stress reactivity, which were examined in terms of peak, activation and regulation slope. Based on previous studies it was assumed that active participants as well as participants engaging in an acute exercise session prior to stress exposure (compared to inactive participants and participants engaging in light stretching before stress exposure) showed reduced stress reactivity and faster recovery for sCort. For sAA, similar hypotheses were adapted, as ANS and HPA systems are known to be mutually dependent and connected at multiple neural levels (Young et al., 2005).

Growth curve modelling with landmark registration was applied and showed habitual exercise activity to evoke a significantly reduced peak sCort release as well as a decreased activation slope and diminishing effects of exercise on sCort regulation. An acute bout of exercise prior to psychosocial stress, however, revealed no significant effects on peak levels, but evoked decreased activation slopes of sCort and sAA and diminished effects on stress regulation for sAA. Detailed consideration of the results on salivary HPA and ANS parameters revealed that stress reactivity was decreased for both, acutely and habitually exercising (i.e. active) participants regarding sCort, whereas only acute exercise evoked this effect in sAA. Consequently, ANS activity might be influenced by acute exercise, but does not seem to be affected by habitual exercise. HPA axis stress response, however, is positively affected by both, habitual and acute exercise, what is also mirrored in results of a recent review (Strahler et al., 2017).

Concerning habitual exercise, the present findings contrast the results of the meta-analysis on cardiovascular parameters conducted by Jackson and Dishman (2006) as well as of the study conducted by Jayasinghe et al. (2017) as both revealed that habitually exercising participants responded higher to a given stressor, but recovered faster. Current results on endocrine data point to the opposite, with both, lower reactions and slower recovery trajectories. This, however, is supported by conclusions of Forcier et al. (2006) for cardiovascular as well as of Klaperski et al. (2014) for cardiovascular and endocrine measures, who both revealed a lower stress reactivity in habitually exercising subjects. Similar to the present findings, the latter study also revealed a prolonged recovery for endocrine parameters in active participants. Taken together, the present results support previous findings (Klaperski et al., 2014; Rimmele et al., 2009, 2007), showing lower stress reactivity, but prolonged recovery. With regard to acute exercise, current results support and extend previous findings (Boutcher and Hamer, 2006; Hamer et al., 2006; Taylor, 2000; Wood et al., 2018; Zschucke et al., 2015), showing reduced stress reactivity and, for the first time, reduced recovery of salivary ANS responses following acute exercise exposure. However, there are still significant variance components in the conditional growth model. Accordingly, adding variables on level 2 would help to explain variability on peak levels as well as on slopes associated with stress reaction and regulation.

The present study accounted for several limitations discussed in

²A manipulation check for the experimental condition can be found in Supplementary Material 1.

Table 2
Results of the unconditional and condition growth models for sCort and sAA.

	sCort				sAA			
	Unconditional growth		Conditional growth		Unconditional growth		Conditional growth	
	Coefficient	p	Coefficient	p	Coefficient	p	Coefficient	p
<i>Fixed effects</i>								
Intercept (peak)	3.986	< .001	4.353	< .001	2.485	< .001	2.564	< .001
Acute Exercise			-0.241	.468			-0.105	.258
Habitual Exercise			-0.092	< .001			-0.008	.350
Habitual Exercise × Acute Exercise			0.072	.076			0.003	.853
Pre-peak slope (activation)	0.059	< .001	0.079	< .001	0.016	< .001	0.022	< .001
Acute Exercise			-0.031	.013			-0.009	.029
Habitual Exercise			-0.002	.044			-0.001	.098
Habitual Exercise × Acute Exercise			0.002	.224			0.001	.148
Post-peak slope (regulation)	-0.030	< .001	-0.036	< .001	-0.026	< .001	-0.031	< .001
Acute Exercise			0.006	.085			0.012	.018
Habitual Exercise			0.001	< .001			< 0.001	.488
Habitual Exercise × Acute Exercise			-0.001	.125			-0.001	.244
<i>Variance components</i>								
σ_e^2 (Level 1)	0.081		0.081		0.013		0.013	
σ_0^2 (Intercept)	1.329	< .001	1.212	< .001	0.104	< .001	0.105	< .001
σ_1^2 (pre-peak slope)	0.002	< .001	0.001	< .001	< 0.001	< .001	< 0.001	< .001
σ_2^2 (post-peak slope)	< 0.001	< .001	< 0.001	< .001	< 0.001	.023	< 0.001	.048

Note: Acute exercise was dummy coded with 0 = placebo exercise and 1 = acute exercise; habitual exercise was entered interval scaled (h/week). sCort: salivary cortisol; sAA: salivary alpha-amylase.

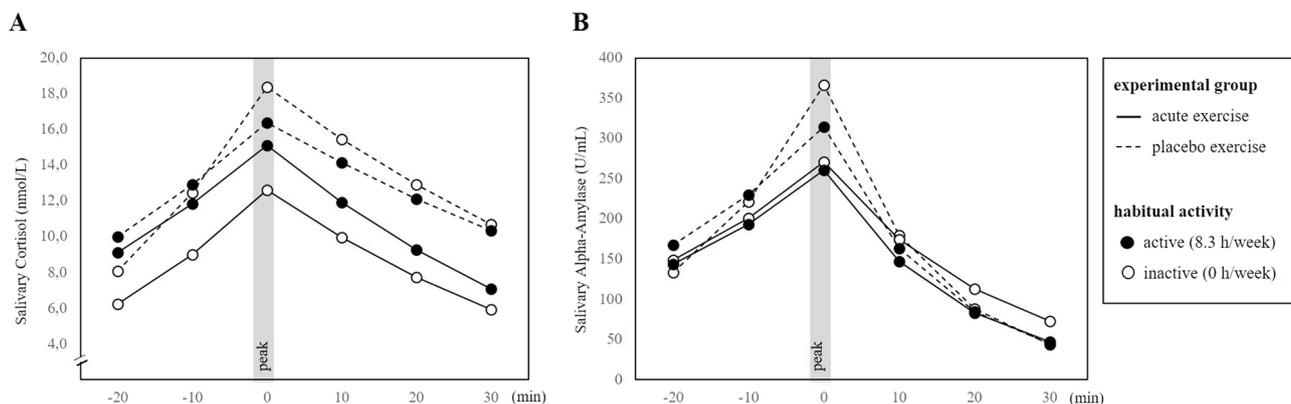


Fig. 4. Prototypical changes in salivary Cortisol (A) and alpha-Amylase (B) for average participants.

previous studies. As compared to Zschucke et al. (2015), the more powerful TSST-G was used, which was shown to be the best stress-evoking laboratory protocol (Skoluda et al., 2015). Most studies in this field suffer from small sample sizes and being underpowered. This was accounted for in the present study by enlarging sample size to 84 participants as well as through the application of a randomized-controlled-trial (RCT) design, as this is considered to be the most powerful experimental design in clinical trials (Stolberg et al., 2004). The current study further adds to existing literature by simultaneously examining the effects of habitual and acute exercise on both major stress response systems. Moreover, the statistical approach accounted for variations in the timing of peak stress responses. Moreover, landmark registration was applied in the current study to strengthen assertions based on statistical analyses for a first time.

Besides these strengths the present investigation also comprised some limitations that need to be discussed. First, evidence presented here is limited to men of similar socio-economic background to ensure comparability of (interindividual) results. Therefore, future studies should also include women and investigate ethnical and socio-demographically diverse samples. Next, inclusion criteria were very strict in order to receive interpretable and commensurable data from saliva samples, so it needs to be considered that external validity is restricted within the present study. Additionally, it should be acknowledged that

participants stated they followed all instructions, but compliance could not be verified objectively. Furthermore, the use of self-report measures for exercise must be seen critically as studies showed an overestimation up to 300%, possibly caused by the need for social desirability and social approval (Klesges et al., 1990). However, the current study design tried to encounter this overestimation by the assessment of individual fitness levels to confirm validity of self-reported exercise amounts.

Albeit many factors were controlled for, several other factors might have affected current results. Studies have shown that cognitive appraisal processes mediate the effect of perceived reaction to social evaluation on sCort responses, and that effects of primary appraisal were attenuated at high levels of perceived reaction during the TSST (Schlotz et al., 2011). Concerning trait measures assessed in the current study, participants scored relatively low on both, trait anxiety and chronic stress. Accordingly, the present results might be attenuated due to range restrictions, as several studies showed the stress-buffering effect of exercise or fitness to be more distinct in samples reporting high stress levels (e.g. Gerber et al., 2013; Klaperski et al., 2012). Moreover, studies showed similar effects for other psychological traits, like mindfulness (Brown et al., 2012), personality traits (Oswald et al., 2006) or rumination (Young and Nolen-Hoeksema, 2001). Consequently, future studies should account for interindividual differences

regarding psychological traits which might have affected current results.

Concerning duration and intensity of the acute exercise session, recommendations from reviews of Hamer et al. (2006) or Gerber and Pühse (2009), for example, were followed. Here, authors concluded that an exercise session needs to last for at least 20 min and to have a minimum intensity of 60% VO₂max. In the current study, the acute exercise session lasted for 30 min and intensity was set at 70% of individual maximum load (in Watts/kg) achieved in the fitness test. Confirming the effectiveness of the exercise protocol, participants rated their perceived level of exhaustion on a Borg rating scale. The aerobic exercise group reported a perceived exhaustion of 16.45, approximately equalling 82% of their individual maximal capacity. As it is known that perceived exertion is correlated to heart rate (Borg et al., 1987; see Supplement Fig. S1) and that heart rate can predict maximum oxygen capacity (Marsh, 2012), it can be assumed that duration and intensity were high enough to elicit meaningful effects.

Another possible concern relies on the temporal distance of the acute exercise session and stressor onset. Raw data from the current study shows that acute exercising participants did not fully recover from the end of the exercise session until stressor onset. However, in the study conducted by Wood et al. (2018), participants went straight from their walk into the TSST-G briefing. However, authors did not measure a baseline prior to the exercise session but referred to the measurement point immediately preceding the TSST-G as a baseline measure. This is why no assumption can be made if these authors had similar findings to those provided in the current study. However, it is merely obvious that fit and unfit as well as walking and non-walking participants differed in sCort concentration at the time of the first measurement. In contrast, in the study conducted by Zschucke et al. (2015), a comparably high amount of time elapsed between exercise and stressor onset (i.e. 90.75 ± 10.73 min). Here, exercising participants did neither show a reduction or elevation in sCort from pre to post exercise, but a reduction from pre exercise to pre stress examination. Hence, participants had enough time to completely recover, with sCort levels falling below baseline level. This makes results difficult to compare for the different studies. As aim of the current study was to examine the direct effect of exercise on stress reactivity, time between exercise and stressor onset was kept as short as possible. This procedure is in line with results revealed by Alderman et al. (2007) who detected a dose–response relationship between exercise intensity and subsequent cardiovascular reactivity to psychological stress, with most attenuated heart rate reactivity within a five minute distance from exercise cessation to stressor onset.

Moreover, some methodological issues need to be raised. First, the temporal resolution of measurement occasions needs to be viewed critically. Although the timing of salivary samples was selected with regard to other studies that applied the TSST (cf., Klaperski et al., 2014), time intervals were not equidistant, leading to difficulties in estimation and interpretation of slopes. In this vein it is possible that broader time intervals of measurements in stress regulation compared to stress reaction might explain the diminished effect of exercise on stress recovery. Second, the current method required an adaption from baseline. As mentioned in the method section, not T1, but T3 (after intervention) had to be used as a baseline to account for intraindividual differences due to the preceding intervention, following the procedure adapted by Wood et al. (2018). Participants, who engaged in acute aerobic exercise, had significantly higher levels in both stress parameters than subject dedicated to the control group. As participants in the intervention group had still not fully recovered from the acute bout of exercise, it was feasible to investigate the immediate effects of acute exercise. The current design also leaves an open question about the possibility that not only exercise, but any other (physical or mental) stressor may have elicited the same response trajectories. Future studies should therefore compare stress responses to exercise conducted at different intensities with response trajectories caused by other stressors

and examine the influence of these patterns on following responses to psychosocial stress situations.

Taken together, the present study made a substantial contribution answering the question about influential effects of habitual and acute exercise on stress reactivity. However, the results revealed several issues that need to be examined in future studies. First, only a small portion of interindividual difference between groups has been explained by habitual and acute exercise. Accordingly, different lines of research should be combined to identify additional possibly influencing mechanisms, like (neuro-)endocrine, biological, behavioural or psychological factors, for example. Moreover, results of Alderman et al. (2007) on the dose–response relationship of intensity and duration of an acute bout of exercise as well as its timely distance to stress exposure should be extended. Additionally, interchangeability with other homo- and heterotypical stressors should be varied and examined to gather further information about apparent differences in stress reactivity. Furthermore, studies should account for the methodological problem of unequally distributed sample intervals and should take equally distributed sample-taking into account.

6. Conclusion

Results revealed significant effects for both acute and habitual exercise on sCort and sAA reactivity due to stress exposure. In particular, habitual exercise showed a significant stress-buffering effect on sCort activation, regulation and peak maximum whereas acute exercise predicted peak levels of this biomarker. In regard to sAA, acute but not habitual exercise predicted response and recovery slopes. In sum, both types of activity evoked benefits for stress reactivity, but only habitual exercise carried on these effects for maximum peak reaction and recovery. The current results confirmed the cross-stressor adaptation hypothesis for habitual exercise. As studies found that chronic hyperactivity of the stress axes and slow recovery have negative impacts on health (Chrousos, 2009), such an adaptation is a central health-protecting mechanism. However, the assumption of acute exercise showing similar stress-buffering effects must be seen critically. Effects were only found in reaction slopes and post peak sAA slope. Therefore, future research should further investigate the stress-buffering and CSA-effects of acute activity, as indications may be used to advice people how to encounter foreseeable stressful events to protect from any stress-related health complaints.

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Conflict of interest

All authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.psyneuen.2019.03.015>.

References

- Alderman, B.L., Arent, S.M., Landers, D.M., Rogers, T.J., 2007. Aerobic exercise intensity and time of stressor administration influence cardiovascular responses to psychological stress. *Psychophysiology* 44 (5), 759–766. <https://doi.org/10.1111/j.1469-8986.2007.00548.x>.
- Babor, T.F., Higgins, Biddle, J.C., Saunders, J.B., Monteiro, M.G., 2001. AUDIT. The Alcohol Use Disorders Identification Test, 2nd ed. World Health Organization.
- Borg, G., 2004. Anstrengungsempfinden und körperliche Aktivität. *Deutsches Ärzteblatt* 101 (15), 1016–1021.
- Borg, G., Hassmén, P., Lagerström, M., 1987. Perceived exertion related to heart rate and blood lactate during arm and leg exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* 56 (6), 679–685.
- Boutcher, S.H., Hamer, M., 2006. Psychobiological reactivity, physical activity, and cardiovascular health. In: Acevedo, E.O., Ekkekakis, P. (Eds.), *Psychobiology of physical activity*. Human Kinetics, Champaign, pp. 161–176.
- Brown, K.W., Weinstein, N., Kreswell, J.D., 2012. Trait mindfulness modulates neuroendocrine and affective responses to social evaluative threat. *Psychoneuroendocrinology* 37 (12), 2037–2041. <https://doi.org/10.1016/j.psyneuen.2012.04.003>.
- Chauhan, E., Bali, A., Singh, N., Jaggi, A.S., 2015. Cross stress adaptation: phenomenon of interactions between homotypic and heterotypic stressors. *Life sciences* 137, 98–104. <https://doi.org/10.1016/j.lfs.2015.07.018>.
- Chrousos, G.P., 2009. Stress and disorders of the stress system. *Nat. Rev. Endocrinol.* 5 (7), 374–381. <https://doi.org/10.1038/nrendo.2009.106>.
- Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol. Bull.* 130 (3), 355–391. <https://doi.org/10.1037/0033-2909.130.3.355>.
- Engert, V., Vogel, S., Efanov, S.I., Duchesne, A., Corbo, V., Ali, N., Pruessner, J.C., 2011. Investigation into the cross-correlation of salivary cortisol and alpha-amylase responses to psychological stress. *Psychoneuroendocrinology* 36 (9), 1294–1302. <https://doi.org/10.1016/j.psyneuen.2011.02.018>.
- Foley, P., Kirschbaum, C., 2010. Human hypothalamus-pituitary-adrenal axis responses to acute psychosocial stress in laboratory settings. *Neurosci. Biobehav. Rev.* 35 (1), 91–96. <https://doi.org/10.1016/j.neubiorev.2010.01.010>.
- Forcier, K., Stroud, L.R., Papanicolaou, G.D., Hitsman, B., Reiches, M., Krishnamoorthy, J., Niaura, R., 2006. Links between physical fitness and cardiovascular reactivity and recovery to psychological stressors: a meta-analysis. *Health Psychol.* 25 (6), 723–739. <https://doi.org/10.1037/0278-6133.25.6.723>.
- Fuchs, R., Klaperski, S., Gerber, M., Seelig, H., 2015. Messung der Bewegungs- und Sportaktivität mit dem BSA-Fragebogen. *Zeitschrift für Gesundheitspsychologie* 23 (2), 60–76. <https://doi.org/10.1026/0943-8149/a000137>.
- Gerber, M., Lindwall, M., Lindegård, A., Börjesson, M., Jonsdottir, I.H., 2013. Cardiorespiratory fitness protects against stress-related symptoms of burnout and depression. *Patient Educ. Couns.* 93 (1), 146–152. <https://doi.org/10.1016/j.pcc.2013.03.021>.
- Gerber, M., Pühse, U., 2009. Review article: do exercise and fitness protect against stress-induced health complaints? A review of the literature. *Scand. J. Public Health* 37 (8), 801–819. <https://doi.org/10.1177/1403494809350522>.
- Gielen, S., Laughlin, M.H., O'Conner, C., Duncker, D.J., 2015. Exercise training in patients with heart disease: review of beneficial effects and clinical recommendations. *Prog. Cardiovasc. Dis.* 57 (4), 347–355. <https://doi.org/10.1016/j.pcad.2014.10.001>.
- Hackney, A.C., 2006. Stress and the neuroendocrine system: the role of exercise as a stressor and modifier of stress. *Expert Rev. Endocrinol. Metab.* 1 (6), 783–792. <https://doi.org/10.1586/17446651.1.6.783>.
- Hackney, A.C., Viru, 1999. Twenty-four-hour cortisol response to multiple daily exercise sessions of moderate and high intensity. *Clin. Physiol.* 19 (2), 178–182. <https://doi.org/10.1046/j.1365-2281.1999.00157.x>.
- Hamer, M., Taylor, A., Steptoe, A., 2006. The effect of acute aerobic exercise on stress related blood pressure responses: a systematic review and meta-analysis. *Biol. Psychol.* 71 (2), 183–190. <https://doi.org/10.1016/j.biopsycho.2005.04.004>.
- Jackson, E.M., Dishman, R.K., 2006. Cardiorespiratory fitness and laboratory stress: a meta-regression analysis. *Psychophysiology* 43 (1), 57–72. <https://doi.org/10.1111/j.1469-8986.2006.00373.x>.
- Jayasinghe, S.U., Torres, S.J., Hussein, M., Fraser, S.F., Lambert, G.W., Turner, A.I., 2017. Fitter women did not have attenuated hemodynamic responses to psychological stress compared with age-matched women with lower levels of fitness. *PLOS ONE* 12 (1), e0169746. <https://doi.org/10.1371/journal.pone.0169746>.
- Katz, D.A., Peckins, M.K., 2017. Cortisol and salivary alpha-amylase trajectories following a group social-evaluative stressor with adolescents. *Psychoneuroendocrinology* 86, 8–16. <https://doi.org/10.1016/j.psyneuen.2017.08.021>.
- Klaperski, S., Seelig, H., Fuchs, R., 2012. Sportaktivität als Stresspuffer. *Zeitschrift für Sportpsychologie* 19 (2), 80–90. <https://doi.org/10.1026/1612-5010/a000061>.
- Klaperski, S., von Dawans, B., Heinrichs, M., Fuchs, R., 2014. Effects of a 12-week endurance training program on the physiological response to psychosocial stress in men: a randomized controlled trial. *J. Behav. Med.* 37 (6), 1118–1133. <https://doi.org/10.1007/s10865-014-9562-9>.
- Klesges, R.C., Eck, L.H., Mellon, M.W., Fulliton, W., Somes, G.W., Hanson, C.L., 1990. The accuracy of self-reports of physical activity. *Med. Sci. Sports Exerc.* 22 (5), 690–697. <https://doi.org/10.1249/00005768-199010000-00022>.
- Knubben, K., Reischies, F.M., Adli, M., Schlattmann, P., Bauer, M., Dimeo, F., 2007. A randomised, controlled study on the effects of a short-term endurance training programme in patients with major depression. *Br. J. Sports Med.* 41 (1), 29–33. <https://doi.org/10.1136/bjsm.2006.030130>.
- Lopez-Duran, N.L., Mayer, S.E., Abelson, J.L., 2014. Modeling neuroendocrine stress reactivity in salivary cortisol: adjusting for peak latency variability. *Stress (Amsterdam, Netherlands)* 17 (4), 285–295. <https://doi.org/10.3109/10253890.2014.915517>.
- Luger, A., Deuster, P.A., Kyle, S.B., Gallucci, W.T., Montgomery, L.C., Gold, P.W., Loriaux, D.L., Chrousos, G.P., 1987. Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *New Engl. J. Med.* 316 (21), 1309–1315. <https://doi.org/10.1056/NEJM198705213162105>.
- Marsh, C.E., 2012. Evaluation of the American College of Sports Medicine submaximal treadmill running test for predicting VO2max. *J. Strength Cond. Res.* 26 (2), 548–554. <https://doi.org/10.1519/JSC.0b013e3181bac56e>.
- Miller, R., Plessow, F., 2013. Transformation techniques for cross-sectional and longitudinal endocrine data: application to salivary cortisol concentrations. *Psychoneuroendocrinology* 38 (6), 941–946. <https://doi.org/10.1016/j.psyneuen.2012.09.013>.
- Mücke, M., Ludyga, S., Colledge, F., Gerber, M., 2018. Influence of regular physical activity and fitness on stress reactivity as measured with the trier social stress test protocol: a systematic review. *Sports Med. (Auckland, N.Z.)* 48 (11), 2607–2622. <https://doi.org/10.1007/s40279-018-0979-0>.
- Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* 34 (4), 486–496. <https://doi.org/10.1016/j.psyneuen.2009.01.014>.
- Nater, U.M., Rohleder, N., Schlotz, W., Ehlert, U., Kirschbaum, C., 2007. Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology* 32 (4), 392–401. <https://doi.org/10.1016/j.psyneuen.2007.02.007>.
- Oswald, L.M., Zandi, P., Nestadt, G., Potash, J.B., Kalaydjian, A.E., Wand, G.S., 2006. Relationship between cortisol responses to stress and personality. *Neuropsychopharmacology* 31 (7), 1583–1591. <https://doi.org/10.1038/sj.npp.1301012>.
- Raudenbush, S.W., Bryk, A.S., Congdon, R., 2011. HLM 7.00 for Windows. Scientific Software International, Inc., Lincolnwood, IL.
- Rejeski, W.J., Thompson, A., Brubaker, P.H., Miller, H.S., 1992. Acute exercise: buffering psychosocial stress responses in women. *Health Psychol.* 11 (6), 355–362. <https://doi.org/10.1037/0278-6133.11.6.355>.
- Rimmele, U., Seiler, R., Marti, B., Wirtz, P.H., Ehlert, U., Heinrichs, M., 2009. The level of physical activity affects adrenal and cardiovascular reactivity to psychosocial stress. *Psychoneuroendocrinology* 34 (2), 190–198. <https://doi.org/10.1016/j.psyneuen.2008.08.023>.
- Rimmele, U., Zellweger, B.C., Marti, B., Seiler, R., Mohiyeddini, C., Ehlert, U., Heinrichs, M., 2007. Trained men show lower cortisol, heart rate and psychological responses to psychosocial stress compared with untrained men. *Psychoneuroendocrinology* 32 (6), 627–635. <https://doi.org/10.1016/j.psyneuen.2007.04.005>.
- Schlottz, W., Hammerfeld, K., Ehlert, U., Gaab, J., 2011. Individual differences in the cortisol response to stress in young healthy men: testing the roles of perceived stress reactivity and threat appraisal using multiphase latent growth curve modeling. *Biol. Psychol.* 87 (2), 257–264. <https://doi.org/10.1016/j.biopsycho.2011.03.005>.
- Schulz, P., Schlottz, W., Becker, P., 2004. Trierer Inventar zum chronischen Stress: TICS; Manual. Hogrefe Verl. für Psychologie, Göttingen, Bern, Toronto, Seattle 61 pp.
- Singer, J.D., Willett, J.B., 2003. *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. Oxford Univ. Press, Oxford, pp. 644.
- Skoluda, N., Strahler, J., Schlottz, W., Niederberger, L., Marques, S., Fischer, S., Thoma, M.V., Spoerri, C., Ehlert, U., Nater, U.M., 2015. Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity. *Psychoneuroendocrinology* 51, 227–236. <https://doi.org/10.1016/j.psyneuen.2014.10.002>.
- Sothmann, M.S., 2006. The cross-stressor adaptation hypothesis and exercise training. In: Acevedo, E.O., Ekkekakis, P. (Eds.), *Psychobiology of Physical Activity*. Human Kinetics, Champaign.
- Sothmann, M.S., Buckworth, J., Claytor, R.P., Cox, R.H.O.N., White-Welkeley, J.E., Dishman, R.K., 1996. Exercise training and the cross-stressor adaptation hypothesis. *Exerc. Sport Sci. Rev.* 24, 267–288. <https://doi.org/10.1249/00003677-199600240-00011>.
- Spielberger, C.D., Gorsuch, R.L., 2010. *State-Trait Anxiety Inventory for Adults: Manual and Sample; Manual, Instrument and Scoring Guide; [Form Y]*. Mind Garden, Menlo Park, Calif. pp. 78.
- Stolberg, H.O., Norman, G., Trop, I., 2004. Randomized controlled trials. *Am. J. Roentgenol.* 183 (6), 1539–1544. <https://doi.org/10.2214/ajr.183.6.01831539>.
- Strahler, J., Mueller, A., Rosenlocher, F., Kirschbaum, C., Rohleder, N., 2010. Salivary alpha-amylase stress reactivity across different age groups. *Psychophysiology* 47 (3), 587–595. <https://doi.org/10.1111/j.1469-8986.2009.00957.x>.
- Strahler, J., Skoluda, N., Kappert, M.B., Nater, U.M., 2017. Simultaneous measurement of salivary cortisol and alpha-amylase: application and recommendations. *Neurosci. Biobehav. Rev.* 83, 657–677. <https://doi.org/10.1016/j.neubiorev.2017.08.015>.
- Taylor, A., 2000. *Physical activity, anxiety and stress*. In: Fox, K.R., Biddle, S., Boutcher, S.H. (Eds.), *Physical Activity and Psychological Well-Being*. Routledge, London, New York, pp. 10–45.
- Thoma, M.V., Kirschbaum, C., Wolf, J.M., Rohleder, N., 2012. Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biol. Psychol.* 91 (3), 342–348. <https://doi.org/10.1016/j.biopsycho.2012.07.008>.
- Trappe, H.-J., Löllgen, H., 2000. Leitlinien zur Ergometrie. *Zeitschrift für Kardiologie* 89 (5), 821–837. <https://doi.org/10.1007/s003920050514>.

- Tsatsoulis, A., Fountoulakis, S., 2006. The protective role of exercise on stress system dysregulation and comorbidities. *Ann. N. Y. Acad. Sci.* 1083, 196–213. <https://doi.org/10.1196/annals.1367.020>.
- van Cauter, E., Leproult, R., Kupfer, D.J., 1996. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J. Clin. Endocrinol. Metab.* 81 (7), 2468–2473. <https://doi.org/10.1210/jcem.81.7.8675562>.
- von Dawans, B., Kirschbaum, C., Heinrichs, M., 2011. The Trier Social Stress Test for Groups (TSST-G): a new research tool for controlled simultaneous social stress exposure in a group format. *Psychoneuroendocrinology* 36 (4), 514–522. <https://doi.org/10.1016/j.psyneuen.2010.08.004>.
- Wood, C.J., Clow, A., Hucklebridge, F., Law, R., Smyth, N., 2018. Physical fitness and prior physical activity are both associated with less cortisol secretion during psychosocial stress. *Anxiety Stress Coping* 31 (2), 135–145. <https://doi.org/10.1080/10615806.2017.1390083>.
- World Health Organization, 2010. *Global Recommendations on Physical Activity for Health*. WHO Press, Geneva, pp. 60.
- Wunsch, K., Kasten, N., Fuchs, R., 2017. The effect of physical activity on sleep quality, well-being, and affect in academic stress periods. *Nat. Sci. Sleep* 9, 117–126. <https://doi.org/10.2147/NSS.S132078>.
- Young, E.A., Abelson, J.L., Cameron, O., 2005. Interaction of brain noradrenergic system and the hypothalamic–pituitary–adrenal (HPA) axis in man. *Psychoneuroendocrinology* 30 (8), 807–814. <https://doi.org/10.1016/j.psyneuen.2005.03.009>.
- Young, E.A., Nolen-Hoeksema, S., 2001. Effect of ruminations on the saliva cortisol response to a social stressor. *Psychoneuroendocrinology* 26 (3), 319–329. [https://doi.org/10.1016/S0306-4530\(00\)00059-7](https://doi.org/10.1016/S0306-4530(00)00059-7).
- Zschucke, E., Renneberg, B., Dimeo, F., Wüstenberg, T., Ströhle, A., 2015. The stress-buffering effect of acute exercise: evidence for HPA axis negative feedback. *Psychoneuroendocrinology* 51, 414–425. <https://doi.org/10.1016/j.psyneuen.2014.10.019>.