Applied relaxation and cortisol secretion: findings from a randomized controlled indicated prevention trial in adults with stress, anxiety, or depressive symptoms

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ABSTRACT

Previous research has shown that relaxation interventions can reduce distress, anxiety, and depression. The exact mechanisms that underlie the efficacy of relaxation interventions remain unresolved. This study aimed to investigate whether applied relaxation (AR) leads to changes in cortisol secretion and whether these effects mediate fewer symptoms due to AR. Data come from a randomized controlled preventive interventional trial (N = 277) with elevated tension/distress, anxiety, or depressive symptomatology. Participants were randomized to an intervention group (IG; n = 139, received AR training), or a non-interventional control group (CG, n = 138). Psychopathological symptoms were assessed with DASS-21 and diagnoses of mental disorders via DIA-X-5. Cortisol was measured as short-term index in saliva (six times/d for 2 d at pre-, post-, and follow-up [FU] assessment) and long-term index in hair samples (once at pre-assessment and FU, respectively). Data were analyzed as pre-specified secondary analyses of the randomized controlled trial (RCT) on completers basis (n = 134 CG, n = 102 IG), using multivariable-adjusted linear regression models and mediation analyses (the DASS-21 change in the IG vs. CG with cortisol (area under the curve [AUC]) as mediator). From pre- to post-assessment, total daily salivary cortisol (AUC) decreased more strongly in the IG vs. CG (β-coefficient: −13.83, 95% confidence interval [CI]: −26.85 to −0.81), but was rendered non-significant when adjusting for pre-assessment AUC. This effect was not found for the cortisol awakening response (CAR) or hair cortisol. There was no evidence for a mediation of cortisol (AUC). These findings provide little support for the idea that cortisol reductions explain the beneficial effects of AR on mental health.

1. Introduction

Mental disorders are highly prevalent and contribute considerably to the disability burden worldwide (Breslau et al., 2017). It is therefore relevant to not only treat but also prevent mental disorders (Mendelson & Eaton, 2018). Mental disorders typically develop gradually from initially transient symptoms to full-threshold mental disorders (Beesdo-Baum et al., 2015). Thus, testing the efficacy of indicated interventions (i.e. in individuals with initial symptoms but no full-threshold disorders yet) promises to not only provide new prevention tools but also to understand the underlying processes of dysfunctional development. Stress plays a crucial role in the development and maintenance of many mental disorders (Schiele et al., 2020). Because relaxation interventions aim to lower psychophysiological tension (Esch et al., 2003), they are promising tools to reduce stress and thus prevent the onset of mental disorders.

A particularly promising relaxation intervention is applied relaxation (AR) – a behavioral coping technique that teaches individuals to recognize initial signs of tension and distress earlier, to react rapidly with relaxation, and to prevent an escalation of tension, distress, and associated symptoms (Öst, 2002). AR has been shown to reduce symptoms in the context of treatment of a variety of clinically manifested somatic diseases and mental disorders (Esch et al., 2003; Jansson et al., 1986; Öst, 1988; Öst & Breitholtz, 2000). In a randomized controlled prevention trial in adults at increased risk for psychopathology, we have recently shown that AR can not only reduce distress, anxiety, and depressive symptoms but also prevent the incidence of (sub-)threshold mental disorders (Beesdo-Baum, n.d.).

In line with these assumptions, previous research has shown that relaxation in general reduced subjective feelings of distress (Öst, 1987) and also psychophysiological tension (Esch et al., 2003). For example, relaxation interventions have been shown to reduce arousal and are linked to subjective stress markers (questionnaires like depression-anxiety-stress scale (DASS), perceived stress scale (PSS), Trier Inventory of Chronic Stress (TICS)), subsequent psychopathology.
(particularly anxiety and depressive disorders (Esch et al., 2002a, 2002b)), and the stress response. Notably, chronic stress was associated with pathophysiology in the immune system (immunosuppression, immunological disease process (Esch, 2002)), cardiovascular system (vasoconstriction, vascular hypertension, endothelial dysfunction, atherosclerosis (Esch et al., 2002a, 2002b)), and metabolic systems (increased catabolism, imbalance in hormonal systems (Esch et al., 2003)). Previous research has further shown numerous beneficial effects of relaxation, especially intervention including changes in cortisol secretion (Dolbier & Rush, 2012; Jensen, 2015; Nickel et al., 2005; Pawlow & Jones, 2002). Individuals with initial distress, and/or depressive, and anxiety symptoms may specifically show a higher cortisol awakening response (CAR) and higher average cortisol levels and relaxation may lower these cortisol indices (Saxbe, 2008). However, the exact mechanisms of the effects of relaxation techniques are not yet clear and there are no studies investigating changes of salivary and hair cortisol indices in relation to AR intervention or their role as mediator of the intervention and prevention efficacy (Manzoni et al., 2008). Previous research on other stress management training techniques including progressive muscle relaxation (PMR) found that pre-session salivary cortisol decreased over the intervention course and that this decline was related to lower subjective distress and negative affectivity as well as more frequent relaxation practice at home (Crues et al., 2000).

Regarding cortisol as the end product of the HPA-axis and therefore a biomarker for the stress response/reduction (Chellew et al., 2015), a wide range of cortisol indices were investigated previously (Fries et al., 2009; Kirschbaum & Hellhammer, 1989; Stalder & Kirschbaum, 2012). In this study, we aimed to differentiate between short and long-term measurement of cortisol, reflecting different time intervals which may be both relevant in detecting changes in the endocrine system following relaxation. Thus, we used saliva as well as hair cortisol indices. Salivary cortisol reflects a short-term measurement of the hormone concentration considering one to 3 d. Hair cortisol measurement represents a putative long-term, cumulative, and retrospective measure of systemic, free cortisol secretion over a period of several months (Chan et al., 2014).

Further, there is evidence that cortisol may mediate the effects of AR on distress, depression, and anxiety symptoms (Adam et al., 2008; Esch et al., 2003). For example, a lower cortisol slope and total daily salivary cortisol were found to mediate the association between sleep and depressive symptoms (Hoyt, Ehrlich, et al., 2016; Hoyt, Bower, et al., 2016), between chronic stress with negative affect and fatigue (Starr et al., 2019), and between high acculturative stress and poorer self-reported health (Garcia et al., 2017). However, no mediation effects of cortisol were also reported (Parent-Lamarche & Marchand, 2018). In sum, findings regarding cortisol as a mediator were inconsistent and rare in non-patient samples but previous studies linked cortisol changes and relaxation interventions (Dolbier & Rush, 2012; Jensen, 2015; Nickel et al., 2005; Pawlow & Jones, 2002).

Using data from the above-mentioned randomized controlled indicated prevention trial that evidenced significant symptom reduction from pre to post (but not follow-up [FU]) as well as reduced incident (sub-)threshold diagnoses following AR intervention vs. usual care control group (CG, Beesdo-Baum et al., submitted), the aim of this study was to examine in a pre-specified secondary analysis of this randomized controlled trial (RCT) (1) whether AR induces favorable physiological changes (decreases) in saliva and hair cortisol secretion, and (2) whether these changes mediate the beneficial effects of AR on psychopathology (i.e. lower symptoms and lower risk of incident mental disorders). We hypothesized that (1) individuals who receive AR training experience a stronger decrease in saliva CAR, total daily salivary cortisol, and hair cortisol from pre- to post-assessment than assessment only controls. Furthermore, we expected that if Hypothesis 1 was to be confirmed, (2) a decrease in CAR, total daily salivary cortisol, and hair cortisol would mediate the efficacy of AR on distress, depressive, and anxiety symptoms as well as on incident psychopathology.

Attention should be paid to the hypothesis that individuals with full-threshold mental disorders will be most likely to show severe dysregulations of the HPA axis (Aas et al., 2019; Beesdo-Baum et al., 2015; Misiak et al., 2020). However, in line with dimensional approaches in clinical psychology, it is plausible to assume that also individuals with elevated psychopathological symptoms but no full-threshold disorders yet (as examined in this study) will show initial dysfunctions of the HPA axis (albeit these may be less severe compared to individuals with full-threshold disorders) (Guerry & Hastings, 2011). The main idea of this prevention study is to examine whether AR training can modify such initial dysfunctions to prevent a further progression to more severe dysfunctions and manifest psychopathologies in the long run. Moreover, it has not yet been resolved whether a dysregulation of the HPA axis only relates to specific disorders (e.g. depression) or constitutes a transdiagnostic marker that characterizes a wide variety of mental disorders and diagnostic classes. However, several studies (Karín et al., 2020; Turner-Cobb, 2005; Young et al., 2021) have associated dysfunctions of the HPA axis activity (as indicated, for example, by changes in the cortisol response) with increased stress, which is relevant in the development of a broad range of mental disorders. In line with transdiagnostic approaches of psychopathology (Stanton et al., 2020), the idea of this study is to examine whether AR training reduces tension and stress and thus leads to favorable changes in HPA axis functioning over time.

2. Methods

2.1. Study sample and procedures

We used data of a randomized controlled indicated preventive interventional trial with one intervention group (IG) and one assessment only CG. Assessments were conducted from 09/2016 to 11/2019 at the Technische Universität Dresden (TUD), Germany. Balanced randomization was based on computer-generated permuted blocks. The IG received AR group training. After completion of the study, CG participants had the possibility to also receive AR group training. Neither participants nor the study personnel were blinded to group
assignment because blinding conditions were not possible due to the psychological-behavioral intervention.

Participants were recruited from the community via flyers, media, and advertisement. After online-prescreening and a personal diagnostic entry exam (see below), three main assessments were conducted, including the pre-assessment, the post-assessment directly after the intervention in the IG and roughly 3 months after pre-assessment in the CG, and a 12-month FU. During all three main assessments, detailed diagnostic information on a range of psychopathological symptoms and associated factors was assessed using self-report questionnaires filled-in on tablet computers in the study center and smartphone-based Ecologic Momentary Assessments (EMAs) in participants’ everyday life (over a period of one week after the respective on-site assessment). Physiological measures (cortisol and heart rate [HR]) were also collected at all main assessments, and standardized diagnostic interviews to assess mental disorders were conducted at study entry and FU (details below). Besides, information on changes in other important psychophysiological indicators, including HR and heart rate variability (HRV) over the course of the study, have also been investigated and findings are presented elsewhere (article currently under revision, Zenker et al.).

Inclusion criteria for the trial were: (1) at least mild levels of tension/distress, anxiety, or depressive symptomatology screened with the DASS-21 Scale (tension/stress score \(>\) 8, anxiety score \(>\) 4, or depression score \(>\) 5), and (2) age between 18 and 55 years. Exclusion criteria were a 12-month DSM-5 diagnosis of a mental disorder assessed with a computer-assisted standardized interview (DIA-X-5, Hoyer et al., 2020), current psychological or psychopharmacological interventions, psychotic symptoms, and acute suicidality. Written informed consent was obtained from all participants after complete study information. The study was approved by the Ethics Committee of the Faculty of Medicine of the TUD (EK 508112015).

From the original sample of \(N=277\) randomized participants, \(n=229\) participants took part in the post-assessment and \(n=225\) participants took part in the FU assessment (see Beesdo-Baum, n.d.). From the \(n=139\) participants who were allocated to the IG, \(n=105\) participants received the intervention. For the current data analyses, in the IG only AR training completer (\(n=105\)) were considered. One participant was excluded due to systemic corticosteroid medication. Participants with missing hair cortisol and missing salivary cortisol data at each wave were excluded (\(n=6\)). Thus, the pre-assessment study sample comprises \(n=236\) participants (\(n=134\) CG, \(n=102\) IG). Due to missing hair cortisol data at pre-assessment and/or FU (\(n=74\), of that \(n=1\) pre-analytical excluded due to value more than three standard deviation (SD) above the mean), analyses with hair cortisol comprises \(n=162\) (\(n=86\) CG, \(n=76\) IG) participants. Due to salivary cortisol data missing at either at pre- and/or post-assessment (\(n=77\), of that \(n=12\) pre-analytical excluded due to value more than three SD above the mean), at post-assessment and/or FU (\(n=95\), of that \(n=19\) pre-analytical excluded due to value more than three SD above the mean), or at pre-assessment and/or FU (\(n=93\), of that \(n=29\) pre-analytical excluded due to value more than three SD above the mean) analyses with salivary cortisol comprise \(n=159\) for pre-to-post analyses (\(n=85\) CG, \(n=74\) IG) participants, \(n=141\) for post-to-FU analyses (\(n=77\) CG, \(n=64\) IG), and \(n=143\) for pre-to-FU analyses (\(n=78\) CG, \(n=65\) IG), respectively (Supplementary Figure 1).

### 2.2. Intervention – applied relaxation (AR)

The AR intervention is based on PMR and followed standardized manual procedures to learn AR initially developed by Öst (2002). Courses were planned to be conducted in groups of 10 persons and were accompanied by psychoeducational elements. Each course was comprised 10 instructor-guided sessions of 60–90 min with the following content: 1) Psychoeducation and AR rationale, PMR (participants learn relaxation with prior tension), 2) release-only relaxation I (participants learn to relax without prior tension), 3) release-only relaxation II, 4) cue-controlled relaxation (participants learn to relax with an individual cue-word), 5) differential relaxation I (participants learn to relax with open eyes and while conducting movements [head, hands, and legs] in sitting position), 6) differential relaxation II (participants learn to relax in different body positions [standing, walking]), 7) rapid relaxation (participants learn to relax in 20–30 sec in different natural environments), 8) AR – imaginal practice (participants imagine a stressful situation and practice rapid relaxation when the first signs of stress are noticed), 9) AR – real life practice (participants learn to rapidly relax whenever they notice first signs of stress in daily life), 10) AR in real life and closing. To accommodate the individual pace of the participants in learning relaxation, each session also included a repetition of previous relaxation exercises. Throughout the training, participants were instructed to fill in a diary on initial physical, cognitive, behavioral, and emotional symptoms in distressing situations as well as to record the success of their daily relaxation practices, which were then discussed within each course session.

### 2.3. Depression-anxiety-stress scale

The 21-item short-form of the DASS-21 (Lovibond & Lovibond, 1995) was used at pre, post, and FU to measure depression, anxiety, and stress symptoms. It includes seven items per scale rated on a 4-point scale (from “never” to “almost always”) during the past two weeks. The DASS-21 total score was generated at pre, post, and FU by summarizing the total values of all three subscales. Differences in DASS-21 scales were calculated using the subtrahend of DASS-21 at post-pre/FU-pre/FU-post, respectively.

### 2.4. Incident mental disorders

Incident DSM-5 mental disorders (first-lifetime incidence or recurrence) were assessed with an updated research version of the fully standardized and computerized Composite International Diagnostic Interview (DIA-X/M-CIDI; Wittchen & Pfister, 1997), applied face-to-face by trained clinical
interviewers [DIA-X-5; Hoyer et al., 2020]). At study entry, symptoms, syndromes, and diagnoses of the following lifetime and 12-month mental disorders were assessed: somatic symptom or related disorders (somatic symptom disorder and illness anxiety disorder); anxiety disorders (panic disorder, generalized anxiety disorder, social anxiety disorder, agoraphobia, and separation anxiety disorder); depressive disorders (major depression and persistent depressive disorder/dysthymia); bipolar disorders (bipolar I and bipolar II disorders); posttraumatic stress and related disorders; substance use disorders (alcohol use disorder, medication use disorders, and illicit substance use disorders). At FU, the same diagnoses were assessed for the past 12-month period. As a 12-month FU is a relatively short time period for the development of incident full-threshold mental disorders (even in a high-risk sample with initial symptoms), we also considered incident subthreshold disorders from study entry until FU (defined as falling short of one diagnostic criterion, e.g. the time criterion).

2.5. Cortisol

Saliva and hair cortisol samples were analyzed in the laboratory of the Biopsychology Unit at TUD, using commercially available chemiluminescence immunoassays with high sensitivity (IBL-International, Hamburg, Germany).

Salivary cortisol: Saliva was collected during EMA, which was conducted over one week in real life after the main on-site assessment visit at pre, post, and FU. On the first two week days of EMA, a study smartphone reminded the participants six times per day to provide saliva samples in parallel with the request to answer questions on the smartphone. Specifically, saliva samples were taken on awakening, 30 min after awakening, at three random time points over the day course, and at bedtime. The three between time points had to be variable (in a predefined range) due to the real-time capturing of questionnaires and time series measurement. Given that these measurements were more valid when participants could not anticipate the exact assessment time; these assessments were created in a random stratified pattern. Participants were instructed to refrain from eating, drinking (except for water), toothbrushing, and smoking 30 min prior to saliva sampling. Participants were instructed to store the samples in Salivette devices (Sarstedt, Rommelsdorf, Germany) frozen at home. MEMS electronically records time of opening and thus allow verifying participants’ sampling adherence. Saliva samples were delivered by participants to the study center and were stored in a laboratory freezer at −80°C. At the laboratory, saliva samples were thawed and subsequently centrifuged for 10 min at 4000 rpm. The intra- and inter-assay coefficients for cortisol were below 7%. The measurement range was between 0.3 and 88.0 nmol/L and the lower limit of detection (LOD, functional sensitivity) was 0.3 nmol/L.

Two salivary cortisol indices were calculated. First, we calculated the CAR by subtracting the awakening from the 30 min after awakening cortisol concentration, divided by the time between these values as measured by the MEMS (Stalder et al., 2016). If CAR time points were not in the range between 20 and 40 min, the CAR was not calculated due to the association between inaccurate post-awakening sampling and underestimation of the CAR (Stalder et al., 2016). Second, we calculated the total daily salivary cortisol level (average cortisol level) as the area under the curve with respect to ground (AUC) using the trapezoid formula (Pruessner et al., 2003). Salivary cortisol concentrations of all six timepoints per day and the time distances between the samples as measured by the MEMS were used. If the time between consecutive samples on any given day exceeded 360 min, this was considered implausible, and the AUC was not calculated. All salivary cortisol indices were averaged across the two sampling days (Adam & Kumari, 2009). After linear transformation, CAR represents the change in cortisol concentration in nmol/L for half an hour after awakening and the AUC represents the average cortisol concentration in nmol/L per hour (Hoyt, Ehrlich, et al., 2016; Hoyt, Bower, et al., 2016). Finally, differences in cortisol levels across the assessments were calculated (subtrahend of cortisol at post-pre/FU-pre/FU-post, respectively).

2.5.1. Hair cortisol

Two hair strands of 3 mm diameter each were cut by research staff as close as possible to the scalp from a posterior vertex position at the participants’ pre-assessment and FU visit at the study center. The proximal 3 cm hair segment was used for analyses, reflecting steroid hormone secretion within 3 months prior to assessment. Details of the pre-analytical procedures were previously described (Stalder & Kirschbaum, 2012). In brief, samples were washed twice in 2.5 mL isopropanol for 3 min. Steroid hormones were extracted from 7.5 mg of whole, non-pulverized hair using 1.8 mL methanol for 18 h at room temperature. 1.6 mL of the clear supernatant was transferred into a new 2 mL
tube.

2.6. Confounder

At pre-assessment visit, height and weight were measured without shoes to the nearest cm. BMI was calculated using the formula weight/height². Physical activity (hours per week of physical activity in the last 3 months), tobacco consumption (yes/no in the last 3 months) and alcohol consumption (from never to two times a day or more in the last 3 months) were assessed with a questionnaire during the hair sampling. Educational status, social class, marital status, and citizenship were assessed via tablet computer. Educational status refers to: low educational status: attendance at main school (secondary school in Germany with lower secondary education, level 2 according to the ISCED), secondary modern school qualification. Middle: high-school diploma, attendance of middle school (type of secondary/junior high school for ages 10–16). High: grammar school in Germany (secondary school ISCED level 3). Other: all other type of school. Marital status was categorized in married/registered partnership, divorced/separated/widowed, and never married. Social class was categorized based on self-report in lowest/lowest middle class, middle class, and upper middle/upper class. Use of corticoid medication or oral contraceptives was assessed with a
medication questionnaire, assessing medication use in the last two weeks, 12 months, and two years. Hair and hair cosmetic-related information including hair color, frequency of hair cleaning, and hair treatment with heat was obtained in a separate questionnaire.

2.7. Statistical analyses

Data were analyzed on a completer basis, including only participants of the IG that completed the AR training with at least four training sessions and participants with complete cortisol data at pre and post, pre and FU, or post and FU. Regarding descriptive analyses, categorical data are given as percentage and continuous data as mean (standard error). 

Following Hypothesis 1, we tested the change (Gu et al., 2018) of cortisol indices (1. CAR, 2. AUC, 3. Hair, A. pre to post, B. pre to FU, C. post to FU) with Mann–Whitney-U-test as well as age- and multivariable-adjusted linear regression models. We used QQ plots to test the normality of regression residuals in linear regression models. Results were presented as β-coefficients with their 95% confidence interval (CI).

Mediation analyses (Hypothesis 2) were planned to be performed using the Stata command medeff (Hicks & Tingley, 2011), implementing age and multivariable-adjusted linear regression models regarding DASS change in the intervention vs. CG and cortisol as mediator. The presented total effect is the effect of the independent variable (group affiliation) on the dependent variable (DASS) without the mediator cortisol. The direct effect is the effect of the independent variable (group affiliation) on the dependent variable (DASS) after considering a mediation (indirect) effect of cortisol. The average causal mediation effect (ACME) is the total effect minus the direct effect. This indirect effect represents the mediation effect. Mediation effects were considered significant when the 95% CI of the ACME and the percentage of the mediated total effect excluded zero. Regarding the incident diagnosis outcome (any incident vs. no incident (sub)-threshold mental disorder), the command medeff included multivariable logistic regression models with any incident diagnosis (yes vs. no) as outcome variable. Results were presented as coefficients with their 95% CI. No odds ratios were presented, as in the Stata mediation analyses the command for a logistic model is logit. Logit fits a logit model for a binary response by maximum likelihood but does not present odds ratios (results are the same) (Stata base reference material, release version 14; StataCorp LLC, College Station, TX).

As presented elsewhere for the total sample (Beesdo-Baum, n.d.), results for the current cortisol completer subsample showed that the DASS total score and DASS depression/anxiety/stress subcale scores were significantly lower in the IG (e.g. post DASS-21 total score: M = 9.10 (SD = 5.77); post DASS depression score: M = 2.13 (SD = 2.20)) compared with the CG (post DASS-21 total score: M = 14.33 (SD = 8.38); DASS depression score: M = 4.28 (SD = 3.49)) at post-assessment, but not at pre-assessment. DASS scores and subscales showed a significantly higher decrease in the IG compared with the CG at pre-assessment to post-assessment (Supplementary Table 1). While symptom improvements were maintained until FU in the IG, CG participants improved considerably from post to FU assessment, so that no significant group differences in DASS-scores were found at FU. However, compared to the CG, the IG experienced a lower risk of incident (sub)threshold mental disorders until FU (IG: n = 34, 39.08%; CG: n = 56, 55.45%).

3.2. Cortisol changes – Hypothesis 1

Means and SD of cortisol measures across assessments are shown in Table 2. The IG and CG did not differ significantly in any cortisol (CAR, AUC, and hair) measure at pre-assessment. However, there was a significant difference between the IG vs. CG in the total daily salivary cortisol change (AUC) from pre to post (p = .02).

Linear regression analyses yielded no consistent significant effects regarding the cortisol change in the IG vs. the CG group from pre to post, from pre to FU, or from post to FU (Table 3). There was only a significant result for total daily salivary cortisol change (AUC change) in the IG vs. CG from pre- to post-assessment in the multivariable-adjusted (mv) model 1 (mv 1: total daily salivary cortisol (AUC), pre to post: β-coefficient: -13.83, 95% CI: -26.85 to -0.81). Regarding
this association, there was no significant result in the age-adjusted model ($\beta$-coefficient: $-10.48$, 95% CI: $-23.02–2.05$) or when additionally adjusting for pre-assessment cortisol levels (mv model 2). We found no effect for salivary CAR change and hair cortisol change. Sex-specific models yielded no significant results (total daily salivary cortisol (AUC), pre to post: men: $\beta$-coefficient: $8.45$, 95% CI: $32.67–15.16$; female: $\beta$-coefficient: $15.48$, 95% CI: $32.07–1.11$) (Supplementary Table 2). As a further result of sensitivity analyses, we found no significant association between the frequency of AR use and cortisol change in the IG (Supplementary Table 4).

### 3.3. Mediator analyses – Hypothesis 2

Cortisol as a mediator for the efficacy of AR on distress, depressive, and anxiety symptoms was only analyzed for total daily salivary cortisol (AUC) from pre to post considering the results of Hypothesis 1. In mediation analyses with changes in the DASS-21 total score from pre to post as outcome, the ACME was not significant (ACME in total daily salivary cortisol (pre to post) models: $\beta$-coefficient: $-0.10$, 95% CI: $-0.54–0.21$). That is, no evidence for a mediation effect was found in this model. The direct and total effect of the DASS-21 change in

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### Table 1. Sample characteristics at pre-assessment for the analysis sample.

|                          | Intervention group ($N=102$) | Control group ($N=134$) | $p^*$ |
|--------------------------|------------------------------|-------------------------|-------|
| Age (M/SD)               | 36.00 ± 10.82                | 33.70 ± 10.15           | .123  |
| Sex                      |                              |                         |       |
| Female                   | 75 (73.53)                   | 91 (67.91)              | .349  |
| Male                     | 27 (26.47)                   | 43 (32.09)              |       |
| BMI                      | 24.25 ± 4.18                 | 24.39 ± 5.23            | .540  |
| Physically active, hours | 2.91 ± 2.80                  | 2.45 ± 2.35             | .351  |
| Oral contraceptives use  | 4 (3.92)                     | 11 (8.21)               | .181  |
| School education         |                              |                         |       |
| Low (8th grade)          | 1 (0.98)                     | 1 (0.75)                | .243  |
| Middle (10th grade)      | 24 (23.53)                   | 27 (20.15)              |       |
| High (12th grade)        | 77 (75.49)                   | 102 (76.12)             |       |
| Other                    | 0 (0)                        | 4 (2.99)                |       |
| Marital status           |                              |                         |       |
| Married/registered partnership | 41 (40.20)    | 45 (33.59)              | .388  |
| Divorced/separated/widowed | 4 (3.92)        | 7 (6.23)                |       |
| Never married            | 57 (55.88)                   | 82 (61.19)              |       |
| Citizenship              |                              |                         |       |
| German                   | 100 (98.04)                  | 132 (98.51)             | .782  |
| Other                    | 2 (1.96)                     | 2 (1.49)                |       |

Data are percentages or means (SE). $^*$Statistical comparisons were performed with $\chi^2$ test (nominal data) or Mann–Whitney-U-test (continuous data).

M: mean; SD: standard deviation; BMI: body mass index.

### Table 2. Cortisol measures at pre-assessment, post-assessment, and follow-up.

|                        | Analysis sample |
|------------------------|-----------------|
|                        | Intervention group ($n=102$) | Control group ($n=134$) |
|                        | Mean SD         | Mean SD      | $p$ Value$^*$ |
| Salivary CAR, nmol/L   |                 |              |              |
| Pre                    | 4.70 ± 7.14     | 2.62 ± 5.50  | .058         |
| Post                   | 2.15 ± 8.41     | 2.26 ± 9.03  | .995         |
| Follow-up              | 2.58 ± 6.58     | 1.89 ± 6.64  | .708         |
| Pre to post            | -3.28 ± 10.75   | -0.26 ± 12.00| .086         |
| Pre to follow-up       | -2.07 ± 9.00    | -1.34 ± 7.48 | .488         |
| Post to follow-up      | -0.02 ± 10.13   | -1.21 ± 10.52| .534         |
| Salivary Total cortisol (AUC), nmol/L |            |              |              |
| Pre                    | 71.65 ± 33.65   | 65.87 ± 34.81| .100         |
| Post                   | 64.74 ± 23.61   | 68.11 ± 32.15| .649         |
| Follow-up              | 64.51 ± 25.68   | 63.04 ± 26.61| .454         |
| Pre to post            | -11.44 ± 37.26  | 0.36 ± 41.05 | .020*        |
| Pre to follow-up       | -10.27 ± 40.31  | -9.45 ± 30.94| .879         |
| Post to follow-up      | -0.49 ± 31.77   | -5.28 ± 36.72| .807         |
| Hair cortisol, pg/mg   |                 |              |              |
| Pre                    | 5.83 ± 6.14     | 4.43 ± 3.22  | .121         |
| Follow-up              | 6.87 ± 7.30     | 5.38 ± 5.65  | .355         |
| Pre to follow-up       | -0.69 ± 6.60    | -0.90 ± 5.09 | .672         |

Statistical comparisons were performed with Mann–Whitney-U-test. A $p$ value $<.05$ was marked with$^*$. SD: standard deviation; DASS: depression anxiety stress scale; CAR: cortisol awakening response. DASS measures are presented in Supplementary Table 1.
the IG vs. CG was significant in models considering total daily salivary cortisol as mediator from pre- to post-assessment (direct effect: $\beta$-coefficient: $-4.24$, 95% CI: $-6.45$ to $-2.21$; total effect: $\beta$-coefficient: $-4.35$, 95% CI: $-6.51$ to $-2.33$). Thus, the DASS-21 change was significant in the IG vs. CG when considering the mediation effect. That is, the improvements in psychopathological symptoms (DASS-21 total score) from pre to post in the IG vs. CG could not be explained by reductions in total daily salivary cortisol.

In the other models considering the DASS change from pre-assessment to FU, and from post-assessment to FU, no significant direct, total, or ACMEs were observed (Table 4). In sex-specific models, the overall estimates remained unchanged (Supplementary Table 3).

In mediation analyses with the categorical incident diagnoses (full- and subthreshold combined) as outcome, similar effects were found: In mediation analyses with incident diagnoses as outcome, the ACME was not significant (ACME in total daily salivary cortisol models from pre to post: $\beta$-coefficient: 95% CI: $-0.001$, 95% CI: $-0.03$ to $-0.02$). Thus, there was no evidence that reductions in total daily salivary cortisol explained the lower risk of incident disorders in the IG vs. CG from entry exam to FU. These mediation analyses models revealed that the direct and total effect on incident cases in the IG vs. CG was significant in models considering total daily salivary cortisol (pre-to-post: direct effect: $\beta$-coefficient: 95% CI: $-0.25$, 95% CI: $-0.43$ to $-0.07$; total effect: $\beta$-coefficient: $-0.25$, 95% CI: $-0.43$ to $-0.08$) as mediator from pre- to post-assessment.

Given that there was no main effect of CAR and hair cortisol with group when examining Hypothesis 1, we did not analyze the role of CAR and hair cortisol as mediators.

4. Discussion

In this pre-specified secondary analysis of a RCT with AR as a preventive intervention in adults with elevated tension/distress, anxiety, or depressive symptoms, there was evidence only for reduced total daily salivary cortisol (AUC) in the IG vs. CG from pre-to- to post-assessment in parallel to significant symptom reduction. There was no evidence for a mediation effect of total daily salivary cortisol (AUC) for the efficacy of AR on distress, depressive, and anxiety symptoms, or the incidence of (sub-)threshold disorders.

4.1. Cortisol changes – Hypothesis 1

AR is known as an effective intervention to reduce symptoms (Esch et al., 2003; Jansson et al., 1986; Öst, 1988; Öst &
Breitholtz, 2000) and it is assumed that relaxation is associated with lower arousal and lower activity of the sympathetic nervous system which may be associated with lower cortisol secretion (Dolbier & Rush, 2012; Jensen, 2015; Nickel et al., 2005; Pawlow & Jones, 2002). Nevertheless, it is important to consider the complex interplay between the relaxation reaction and physiological outcomes. In this context, the presented results add to the inconsistency in previous literature (review of RCTs (Ryan et al., 2016)) on preventive interventions on cortisol indices and are in line with studies providing no consistent evidence for effects on cortisol regarding relaxation (Klatt et al., 2009; Limm et al., 2011; Richter et al., 2012). Considering the total daily salivary cortisol level, a reduced average cortisol level was found after participation in relaxation intervention (Chellew et al., 2015). We only found stronger decreases in total daily salivary cortisol but no other cortisol indices in the IG vs. CG, and only from pre-to-post assessment and not from pre-to-FU or post-to-FU assessment. Although such a select finding must be interpreted with great caution, it should be noted that it matches with the core symptom-related findings of our randomized controlled trial. As previously reported elsewhere (Beesdo-Baum, n.d.), we found significant DASS symptom decreases in the AR IG relative to the CG from pre-to-post assessment, with no group differences at FU due to significant (spontaneous) improvements after post assessment in the CG. Thus, one might speculate that the accelerated symptom improvement goes along with parallel measurable effects on a physiological level as indicated by reduction of total daily salivary cortisol and both are attributable to AR. The lack of long-term group differences is largely explainable by spontaneous improvements in the CG, rather than a worsening in the IG. As discussed elsewhere (Beesdo-Baum, n.d.), our indicative target group of adults with mild symptomatology but no threshold mental disorder may have not been severe enough at pre-assessment, or the FU period may not have been long enough to demonstrate larger, more consistent, and more pervasive effects on cortisol indices. The significance of the total daily salivary cortisol change in IG vs. CG was attenuated in the multivariable model taking the pre-assessment cortisol level into account; further demonstrates the need for replication, ideally in larger and possibly more severe samples. Our finding of lowered incidences of subthreshold mental disorders in the intervention relative to the CG did not reflect in cortisol group differences at FU. This might be explained by the typical “waxing and waning” of mental disorders (Beesdo-Baum et al., 2015). Incident cases during the FU might have been improved or remitted again by the time of the FU assessment and thus not reflect in higher cortisol indices.

Our study showed an effect of AR particularly on the total daily salivary cortisol index but not on CAR. A possible explanation might be that CAR may reflect an anticipated necessary reaction to the demands of the approaching day (Chellew et al., 2015), which is not directly affected by AR. In contrast, total daily salivary cortisol might be correlated with routine AR use due to the aim of AR to decrease overall daily tension/distress by interrupting the cycle and preventing the escalation of stress symptoms. Thus, practicing AR frequently may lower the overall basal cortisol secretion throughout the day and thus total daily salivary cortisol levels.

In contrast to our findings, some previous studies with highly different designs and methods showed evidence of a steeper cortisol slope in mindfulness-based intervention, and of an association between cortisol and psychosocial health (Adam et al., 2017). In addition, a lower CAR has been linked to psychosocial interventions in numerous studies (Jensen, 2015; Richter et al., 2012) with, however, different methodology reading specific study populations, the inclusion of stress tests, and less control of post-awakening cortisol secretion compared to this study. Further studies found no effect of stress management on the CAR and average cortisol levels (Klatt et al., 2009; Limm et al., 2011; Richter et al., 2012). The present results for CAR may indicate that the participant’s anticipated demands of the day which are associated with morning cortisol secretion (Adam, 2006) did not differ during and after learning and training AR. It may be that participants need more time for intense training and practice to strengthen their confidence in their own higher stress resilience before they anticipate a more relaxed attitude on daily demands in general which may be also seen in the CAR.

Regarding the null findings for CAR and hair cortisol, we consider two main reasons for the present null-findings. First, we used a sample of generally healthy participants, indeed with psychopathological symptoms, but without a 12-month diagnosis of a mental disorder. Thus, it is assumed that participants had no substantial HPA axis dysregulation at pre-assessment, which might result in floor effects and lack of demonstrable group differences. However, reading the inclusion criteria of initial distress, anxiety, and depressive symptoms, subclinical manifestations with physiological changes may be likely as participants were not completely symptom free. Further, it may be that participants with a developing clinical diagnosis before FU show increasing cortisol concentrations. According to our hypothesis, this would be seen in cortisol differences between IG and CG, as fewer incident cases of mental disorders were anticipated in the IG. However, we did not observe this effect in our study. Generally, the effects on cortisol might be (more) visible in more severely affected individuals, or even only in patient samples. To demonstrate intervention and prevention effects on a physiological level, it might be necessary to include indicators of HPA axis dysregulation among the inclusion criteria for a study. Indeed, there is evidence for cortisol as a predictive marker for clinical depression (Caparros-Gonzalez et al., 2017; Kische et al., 2018), but preventative effects in the context of participants without previous diagnoses might be more difficult to demonstrate in general. This may also be influenced by the fact, that especially depressive symptoms may be based on a trait vulnerability rather than a state effect of endocrinological factors (Mocking et al., 2015). Cognitive models assume coherence between different stress aspects like physiologic and psychologic levels of analysis, although, conflicting findings exit (Campbell & Ehler, 2012) and meta-analyses found only weak association between subjective distress and cortisol response (Campbell & Ehler, 2012; Dickerson & Kemeny, 2004). The complexity of the HPA-axis may exacerbate the analyses of direct findings, as a
part of experienced stressors are associated with the cortisol response but independent of the reported subjective stress (Dickerson & Kemeny, 2004; Starr et al., 2019).

A second explanation for our null or inconstant findings regarding cortisol changes is the statistical power for this analysis. Power calculation was performed regarding the primary outcome measures of this randomized controlled trial (DASS-21 and incident diagnoses). Thus, false-negative results due to low statistical power for effects on cortisol cannot be ruled out (Höfler et al., 2018).

4.2. Cortisol as mediator – Hypothesis 2

After testing Hypothesis 1, we found that the conditions for a mediation of cortisol were not met for CAR and hair cortisol (Baron & Kenny, 1986). Thus, we only considered total daily salivary cortisol (AUC) change from pre to post as a mediator.

As we showed previously, AR is an effective preventive intervention resulting in accelerated symptom improvement and reduced incidences of (sub-)threshold mental disorders (Beesdo-Baum, n.d.). The current analysis showed that these findings hold true when considering cortisol in mediator models. However, there was no evidence of a mediator effect of cortisol in this RCT. Hypothesis 2 was based on hypothesis-generating and epidemiological research, suggesting that one to seven percent of variance in psychosocial variables might be explained by diurnal cortisol secretion, a mediating effect of cortisol regarding relaxation (Adam et al., 2008; Esch et al., 2003), positive associations between stress cortisol secretion, and psychopathological symptoms (McEwen & Wingfield, 2003). Previous findings in patients (Hoyt, Ehrlich, et al., 2016; Hoyt, Bower, et al., 2016) or in the general population with focus on stressors (in contrast to report of stress) (Garcia et al., 2017; Starr et al., 2019) found evidence for cortisol as mediator. However, no previous study examined the mediating role of cortisol for AR intervention in a RCT and no direct comparison can be drawn. In general, it may be difficult to replicate findings from clinical studies, based on the observation that only in about half the cases could RCT studies based on the general population confirm results from RCTs with a clinical sample (Ryan et al., 2016). Further, studies in other preventive contexts have shown that cortisol mediates effects only in participants with difficult psychosocial background (O’Neal, 2010), but this cofactor was not assessed in this study. In sum, the present negative mediating results indicate that AR may work mainly by alternative mediating mechanisms (Ryan et al., 2016) in non-patient samples. This may include affectivity, self-efficacy, internal control beliefs, and favorable cognitive and behavioral coping (Beesdo-Baum, n.d.; Cruess et al., 2000).

4.3. Strengths and limitations

The strengths of this study are the RCT design, the valid cortisol measurement following recommendations, and the use of standardized diagnostic interviews for ruling out and establishing DSM-5 based diagnoses. Limitations might have arisen from lack of detailed control for awakening time and lack of data in regard to menstrual cycle status. Further, mediation analyses cannot be interpreted causally, as mediators and dependent variables were assessed at the same study time point. For causal mediation analyses, the mediator must be assessed between the intervention and the dependent variable (Baron & Kenny, 1986). An additional limitation was the hormone measurement via immunoassay, instead of using liquid chromatography-tandem mass spectrometry. All data concerning symptomatology are based on self-report without clinician evaluations which may be subject to bias. Further limitations were the lack of information regarding adherence to behavioral restrictions prior to saliva sampling and the three variable timepoints during saliva sampling, making it difficult for the participants to follow restrictions. The lacking adherence data may have led to unidentifiable confounded salivary cortisol data. This study had a relatively large sample size, but nevertheless, statistical power for cortisol effect was low. Initially, statistical power was calculated for main effects in the primary analyses of the RCT (a priori analysis for statistical power). For cortisol analyses, statistical power could only be calculated as post hoc analyses, which were not recommended (Grant et al., 2018; Levine & Ensom, 2001). Regarding the low statistical power, even larger studies are needed to examine potentially consistent effects on cortisol and the mediation effects with high statistical power. In sensitivity analyses, we used the question how often AR training was conducted during the last week and investigated the association with cortisol change. This variable only depicted the last week, however, the drastic increase in relaxation applications in the IG vs. CG (at post: IG: mean 4.79 (SD 4.46), CG: mean 0.68 (SD 1.12)) demonstrates a successful treatment check. Finally, based on this study, no conclusions can be drawn about the immediate short-term effect of AR, as we did not assess salivary cortisol directly before and after every AR session, or before and after each AR application in real life.

5. Conclusion

Results of this preventive RCT yielded some evidence for reduced total diurnal salivary cortisol (AUC) after AR intervention in participants in the IG vs. the CG parallel to symptom reduction. Given that we found no main effect for CAR or hair cortisol, our results underline that existing associations between cortisol and symptoms of stress/distress, depression, and anxiety in clinical settings may be weak in preventive settings. Additionally, we found no evidence for a mediation effect of total diurnal salivary cortisol (AUC). At this, favorable mediating effects for AR on mental health might be due to other mechanisms, most likely psychological, and mainly independent of cortisol. To further elucidate consistency of effects on cortisol and the role of cortisol in the effectiveness of preventive relaxation intervention, larger longitudinal RCTs are needed with especially repeated and continuous cortisol measurement.
Acknowledgments

The authors thank Dr. Michael Höfler for his valuable advice regarding statistical analyses. The authors thank Max Jacob and all the research assistants and study participants for their support.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Clinical trials registry

The trial has been registered at ClinicalTrials.gov (NCT03311529): https://clinicaltrials.gov/ct2/show/NCT03311529

Funding

The EASY study (Effectiveness and underlying mechanisms of AR as indicated for preventive intervention in subjects at increased risk for mental disorders) has been funded by the Deutsche Forschungsgemeinschaft (DFG) project no. AS 497/1-1. Ms. Zenker, Dr. Asselmann, and Dr. Beesdo-Baum report grants from the Federal Ministry of Education and Research (BMBF) during the conduct of the study. Dr. Beesdo-Baum reports grants from the Deutsche Forschungsgemeinschaft (DFG), during the conduct of the study. The trial has been registered at ClinicalTrials.gov (NCT03311529): https://clinicaltrials.gov/ct2/show/NCT03311529

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