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Effects of a mindfulness-based intervention on mindfulness, stress, salivary alpha-amylase and cortisol in everyday life

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Abstract
A large body of literature has shown the effectiveness of mindfulness-based interventions (MBI) on stress-reduction. However, little is known about their effects on psychobiological stress-markers in daily life through an ecological momentary assessment approach. Our study examines the effects of MBI on state mindfulness, perceived stress, and indicators of sympathetic-nervous-system (saliva alpha-amylase, sAA) and hypothalamic-pituitary-adrenal-axis (saliva cortisol, sCort) activation in daily life. Twenty-eight individuals participated in a three-month MBI (IG) and were compared to 46 controls (CG). An ecological momentary assessment (EMA) was used to assess mindfulness, stress, sAA and sCort at six measurements per day on two days each before and after the MBI. Multilevel-modeling was used to analyze the data on a moment-to-moment and averaged day-level. The IG showed decreased sAA levels (AUCg) from pre to post, while the CG showed increases. Furthermore, diurnal decreases in sCort (AUCi) were pronounced in the IG compared to the CG. On a momentary basis, mindfulness was associated with lower stress and sAA levels, but not sCort. As such, we show that MBI can reduce sympathetic and to a lesser extent hypothalamic-pituitary-adrenal activation in daily life. Increased mindfulness can momentarily decrease stress and stress-related autonomic activation with implications for health. Our results emphasize the importance of brief interventions that can be easily integrated into everyday life.

KEYWORDS
alpha-amylase sAA, cortisol sCort, ecological momentary assessment EMA, mindfulness, multilevel-modeling, stress

1 | INTRODUCTION

While a stress-response in the face of acute challenges is widely regarded as healthy and functional, permanent exposure to stressors and the failure to recover from them can lead to a dysregulation of the neurobiological stress systems (Schneiderman et al., 2005). Serving as a major stress-responsive system, the sympathetic nervous system (SNS) enables a fast reaction to (psychosocial) stressors, for example, by influencing both the cardiovascular and respiratory systems as well as muscle tonus. It is especially sympathetic dominance over parasympathetic activation associated with detrimental health effects (Charmandari et al., 2005). As one outcome of SNS activity, the enzyme alpha-amylase seems to play an important role in the cascade of acute stress-response processes (Ditzen et al., 2014; Rohleder et al., 2006). Saliva alpha-amylase (sAA) reacts very sensitively to stress-induced changes in the SNS (e.g., Nater et al., 2005).

Moreover, the hypothalamic pituitary-adrenal axis (HPA) stress system helps the body adapt to stressors and respond adaptively at the behavioral level in concert with but at a slower pace than the SNS. Through the steroid hormone cortisol released from the adrenal glands about 15–20 min after initiation of a stressor, the HPA-axis has short and long-term effects on metabolic and immune functioning. Besides its immediate response to stress, the HPA-axis shows a strong diurnal dynamic with accentuated increases after awakening and slow but continuous decreases throughout the day. It is this decrease, which has been related to mental and physical health in recent studies (e.g., Hoyt et al., 2015).

Although stress can have long-term detrimental consequences on a behavioral level, coping strategies can successfully counteract stress and its consequences. A variety of mind-body interventions have shown to prevent diseases, clinically reduce symptoms and/or mitigate their consequences. Mindfulness-based techniques belong to the most prominent coping strategies to handle stress (reactions) to date. While these do not fall into the category of relaxation techniques, they aim to direct attention to the here-and-now-experience (via focused attention or open-awareness; see Lutz et al., 2008). Combined with a non-judgmental and accepting attitude toward the qualitative content of the experience—regardless of whether the experience is pleasant, neutral or unpleasant—they are not aiming to actively change it. One consequence of this kind of new reference to the present-moment-experience—especially acceptance—is relaxation and overall stress resilience (Chin et al., 2019). The most well-known and extensively researched mindfulness intervention programs are the Mindfulness-Based Stress-Reduction (MBSR) (Kabat-Zinn, 2003) and the Mindfulness-Based Cognitive Therapy (MBCT) (Segal et al., 2013). Both include an 8-week group program with the goal of using formal and informal mindfulness meditation techniques—including mindfulness breathing, various sitting and walking meditations, yoga exercises, body scan etc. These techniques increase the ability to be mindfully aware of the present moment, de-automatize and non-react, de-identify with certain thoughts and feelings by means of an increased self-regulating skill. Moreover, another important part of mindfulness is attention regulation characterized by continuously redirecting the attention to the chosen object (i.e., just observing) while improving the ability to inhibit the tendency to follow any kind of a given distraction and act more or less automatically (for an overview of the underlying working mechanisms see Lindsay & Creswell, 2019; Tang et al., 2015).

Several research studies convincingly exhibit the effectiveness of a number of mindfulness-based interventions (MBIs) with average medium effect-sizes (e.g., Khoury et al., 2015) on well-being and quality of life (Godfrin & van Heeringen, 2010). MBIs reduce stress (Gotink et al., 2015) and a range of psychological symptoms, including rumination (Perestelo-Perez et al., 2017), depression, anxiety (e.g., Blanck et al., 2018), and others (Goldberg et al., 2018).

In addition, mindfulness is associated with the activity of psychobiological processes. One aspect of meditation practice particularly associated with altered physiological responses is breathing practice (Pascoe & Crewther, 2016). Overall, however, the *integrated specificity model* can explain the relationship between the different ingredients of mindfulness and physiological modification through the altered cognitive appraisal of negative stimuli and their associated emotions: Mindfulness leads to a reduced negative affect associated with not automatically appraising stressors as threatening. This, in turn, leads to a change in the resulting integrated pattern of physiological responses, for example, cortisol responses are attenuated (Denson et al., 2009).

In two meta-analyses by Pascoe, Thompson, Jenkins, et al. (2017, Pascoe et al. (2017), MBSR or mindfulness were examined in different samples and were associated with a reduction in cortisol-values (see also Sanada et al., 2016) and TNF alpha values, which highlight a positive effect at the physiological level. However, it had no effect on HRV, whereas MBI, including Yoga, did. Furthermore, we found that MBI altered epigenetic serotonin transporter related mechanisms (Stoffel et al., 2019).

In addition, sympathetic markers, such as sAA, are interpreted as outcomes in connection with MBI: A meta-analysis addressing workplace-based mindfulness indicated improvements in SNS reactivity by reduced sAA post-intervention (Heckenberg et al., 2018); this was also found in a surgical intensive care unit (Duchemin et al., 2015), in veterinary students performing surgery (Stevens et al., 2019) and in cancer survivors (Lipschitz et al., 2013).

Furthermore, heterogeneous effects of different MBI at the physiological level as a result of acute stress induction in the laboratory (induced by the Trier Social Stress Test) have only recently been demonstrated. About half of the studies that measured cortisol responses showed a stress-buffering effect of MBI compared to respective control groups, whereas only one out of three studies that investigated alpha-amylase detected a stress-buffering effect of MBI (Morton et al., 2020). To date, though, we are not aware of any significant study that has examined the physiological (interaction) effects on a momentary level in everyday life.

Overall, those with severe impairments (Khoury et al., 2013) show the strongest psychological improvement following MBIs. Medical students belong to a particularly vulnerable group due to high time and achievement pressure. They report high stress rates and are likely to suffer from psychopathological symptoms (Rotenstein et al., 2016). At the same time, medical students could particularly benefit from interventions that strengthen personal resources such as mindfulness (Heinen et al., 2017).

The positive changes in MBIs usually relate to an increase in trait mindfulness (Baer et al., 2006). But what about *state* mindfulness? Although studies suggest an increase in both trait and state mindfulness as a result of MBI (Kiken et al., 2015), it appears that trait and state mindfulness seem to be two interrelated but different constructs (Thompson & Waltz, 2007). *Trait* mindfulness refers to a general tendency or (stable) personality trait that can be more or less pronounced or can also be increased in the medium and long term through training. The individual's level of mindfulness at a given point in time circumscribes a *state* of experienced mindfulness (Medvedev et al., 2017). The state description involves a current person-occasion interaction. Thus, it is significant to measure the state of mindfulness in the face of certain stressful circumstances in everyday life. Inconsistent findings exist regarding the association of trait and state mindfulness (Tanay & Bernstein, 2013), ranging from no correlations to overlaps in only a few sub-dimensions of the Five Facet Mindfulness Questionnaire and state mindfulness during meditation (Bravo et al., 2018).

Compared to single self-report measures at a given time, an ecological momentary assessment (EMA) is of particular value due to its ecological validity and relevance for real-life phenomena (Conner & Barrett, 2012). In a study comparing traditional paper-pencil with EMA assessment pre-post intervention, results showed significant improvements of mindfulness and reduction of depressive symptoms and anxiety only when EMA was used (Moore et al., 2016). Overall, EMA studies have revealed positive effects of mindfulness on subjective stress and emotion regulation (Bai et al., 2020). Regardless of the severity of the threat and the emotional state, present awareness enabled adaptive stress responses in daily life (Donald et al., 2016). In another study, mindfulness particularly enhanced serenity compared to induced rumination, and state mindfulness was associated with somewhat increasing positive valence over that day (Huffziger et al., 2013). There have been some EMA-based studies in both the field of mindfulness and in the field of psychobiology, but these two have rarely been studied in combination. Beyond that, no study to date has considered an EMA-based approach evaluating a mindfulness-based intervention in combination with psychobiological markers.

So far it has not been tested whether mindfulness-training might improve HPA-axis and SNS outcomes in everyday life utilizing an EMA approach. This association bears particular relevance because SNS outcomes show immediate reactivity and high fluctuation in response to a broad range of daily stressors.

We aimed at investigating whether a three-month MBI increases state mindfulness, buffers subjective stress and HPA-axis and sympathetic activation in response to relevant stressors in daily life—which could indicate a particular relevance in contrast to results based on pre-post laboratory measurements. We chose an EMA conceptualization of state mindfulness, perceived acute stress, sAA and sCort trajectories during daily life in medical students preparing for a major exam. Specifically, we expected that higher state mindfulness would be associated to lower sAA, lower sCort, and lower perceived stress on a daily or moment-to-moment basis.

2 | METHOD

2.1 | Participants

A total of 74 medical students (third semester), at the end of the term, were about to take their first major exam and participated in our study. Recruitment took place at the Medical School of Heidelberg University, Germany. All third semester medical students were enlisted in mandatory elective courses in medical psychology.

The assignment to the intervention (IG) and control group (CG) was based on the course content and the voluntary agreement of the students to participate in the study. Those who attended a course on stress and stress management—including mindfulness- based practices—defined the IG, while those who attended courses without relation to stress management defined the CG (see below for further details).

For the main outcome parameters, such as the AUC's as well as changes in perceived stress and mindfulness, we aimed at detecting a moderate sized effect ($>f = 0.25$) (see Khoury et al., 2015) between the IG and CG in a repeated measures design with $k = 24$ measurements (momentary assessments in everyday life), and a correlation between repeated-measures of $r = 0.35$, $\alpha = 0.05$, and $(1-\beta) = 0.8$ (between factors repeated measures ANOVA). However, since the main analyses were conducted using multilevel models (MLM) and, this power analysis can also be seen as approximation. Yet, it is nearly impossible to find comparable research which defines all necessary parameters to conduct power analyses for MLM directly. For statistical power, the total number of observations (i.e.,: participants × days × measurement points per day) is more important than the sample size alone (for details see Bolger et al., 2012). Therefore, to make sure that the results of the MLM were not confounded by biased estimates of model coefficients, standard errors, or variance components, standard simulation work was consulted which indicates that a sample size of $N = 50$ on level 2 allows for reliable estimates in such models (Maas & Hox, 2005). Correspondingly, the analyses via G × Power revealed a minimum sample size of $N = 50$. Thus, a minimum of $n = 25$ participants for each group had to be recruited.

All courses covered a period of three months. In the IG, $N = 28$ ($n$female = 18; 64.3%; $n$male = 10; 35.7%) and in the CG, $N = 46$ individuals ($n$female = 30; 65.2%; $n$male = 16; 34.8%) with a Mage of 21.1 ($SD = \pm 2.3$) participated. Data on sex, age, ethnicity, as well as health-related behavior and status (such as the body-mass-index, BMI) and current medication-intake were assessed via self-report and interviews.

Participants were excluded from the statistical analysis if they stated to have a chronic, severe somatic disease (e.g., diabetes, cancer, history of heart-attacks); and/or a severe psychiatric diagnosis (e.g., schizophrenia, a neurodevelopmental disorder, a recurrent affective disorder etc.). Furthermore, heavy smoking of more than >20 cigarettes per day, a history of or current substance abuse as well as the prolonged intake of psychiatric medication were regarded as exclusion criteria in addition to pregnancy. Only one person of the IG was excluded from analysis due to psychiatric medication but participated in the intervention, while for all other participants none of the defined exclusion criteria were recorded via self-report.

2.2 | Procedure

A longitudinal design with an EMA approach was used with two consecutive days of data collection pre- and post-intervention. Sample size calculations, including the number of samples and days, were guided by the standard recommendation of using more than one day for assessment (Adam & Kumari, 2009; Stoffel et al., 2021). To assess diurnal rhythms of alpha-amylase and cortisol as well as their associations with mindfulness and stress, we chose a combination of an event-based and a time-based assessment (Shiffman et al., 2008), thus, forming an event-related fixed-occasion design (Kudielka et al., 2012). Within this framework, we used a medium intensity protocol with a total of six sampling occasions throughout the day. Such sampling protocols were shown to provide valid representations of diurnal secretion of psychobiological stress-parameters while reducing participant burden and costs to a minimum (Hoyt et al., 2016). For our within-subject comparisons of for example, associations of sAA and state mindfulness in everyday life, each participant provided up to 24 data points (six on a total of four days; saliva samples as well as self-reports of stress and mindfulness), which sums up to a total of 1,776 measurements.

The first survey around the time of awakening triggered the following five sampling times, which took place 30, 150, 480 and 720 min thereafter. The last measurement took place at bedtime. At each time, participants received a text message with a link leading to the internet-based assessment. Participants first provided the saliva sample and then answered a questionnaire to report on control (e.g., eating, see Strahler et al., 2017), and outcome parameters.

The study was approved by the ethics committee of the Medical Faculty of the University, Heidelberg, Germany (S-355/2015) and registered at the German Clinical Trials Register (DRKS00013950). Prior to participation, all participants provided written informed consent. In case of full participation, subjects received a monetary compensation (80 €).

2.2.1 | Mindfulness-based intervention MBI

The Mindfulness-Based intervention was offered as part of a regular elective required course offering for undergraduate medical students. Students have the option to freely choose between different course content in the 3rd semester (e.g., physician-patient communication; focus on palliative or family practice settings, etc.). All students who chose the mindfulness-based stress intervention course had the opportunity to also participate in the study on a voluntary basis. However, agreeing or declining to participate in the study did not affect the course experience or course participation. The course was offered independently of the study to all students who had freely chosen the course as part of their academic pursuits.

The course included active application of mindfulness-based stress reduction techniques and covered the following sequence in a three-month period: a one-day theoretical introduction, four three-hour evening-sessions every two weeks and a final two-hour-session at the end of the intervention period. Each session included a theoretical introduction to biological stress-systems and relevant issues of mindfulness-related stress reduction techniques and their effects on genes, immune-functioning, neurobiological changes etc. This psychoeducation part was followed by a practical part with instructed contemplative exercises and a moderated inquiry for reasons of perspective taking and socially connecting among participants. Three practices were introduced, instructed and practiced: (1) Mindfulness meditation on the breath while sitting, (2) body scan as well as (3) progressive muscle relaxation. These three practices were chosen as focal points because, as explained above, on the one hand, they are considered easily accessible and implementable, the involvement of the body favors the reference to the present moment-experience; on the other hand, attention control increases self-regulating skills, the practice of mindful breathing and active muscle relaxation can particularly influence psychobiological parameters.

Audio material was provided for the participants to practice at home on a voluntary basis.

An experienced body- and health counselor holding a Ph.D. in biology led the courses in which the intervention was conducted.

2.3 | Measures

2.3.1 | Psychobiological measures

SaliCaps® (IBL, Germany) were used for the collection of saliva via the passive drool technique. Participants were asked to store their saliva samples in their refrigerators until they returned them to the study personnel at the end of the week. Samples were frozen at −80°C until analysis for no more than six months.

sAA was analyzed using a commercially available kinetic colorimetric kit with reagents from Roche (Roche Diagnostics, Germany). sCort was measured using an enzyme- linked immunosorbent assay (ELISA; RE52611, IBL International, Germany). All samples were determined in duplicates and mean values were used for statistical analysis.

The intra-assay coefficient of variation (CV) was 5.08% for sAA and 7.39% for sCort.

The inter-assay CV was 7.09% for sAA and 6.06% for sCort. The areas under the curve (AUCg and AUCi) were calculated for sAA and sCort using formulas obtained from Pruessner et al. (2003). While the AUCg indicates the total output of the neuroendocrine system of interest within one day, the AUCi is an indicator of change with lower values indicating a stronger down-regulation of the system on a given day.

2.3.2 | Self-report measures

sMAAS

The state Mindful Attention Awareness Scale is composed of 5 items and assesses the current experiences with mindful attention. In its original English version, it has shown very good psychometric properties with internal consistencies of 0.92 and correlations with psychological well-being (Brown & Ryan, 2003). No data on psychometric properties for the German version are available yet. Response options ranged from 1 = "not at all" to 7 = "very much". All items had to be reversed, as they are formulated in a negative or indirect way,for example, "I was preoccupied with the future or the past." We computed mean scores with higher scores reflecting higher state mindfulness. In our study, the German version reveals an internal consistency of $\alpha = 0.95$.

Perceived stress

Stress currently perceived was assessed using a visual analogue scale asking (Gaab et al., 2005; Shields, 2020), "Please indicate how you feel at the moment", ranging from 1 = "stressed" to 100 = "relaxed". To enhance interpretability, the item was reversed before conducting statistical analyses.

2.4 | Statistical analyses

Multilevel models (MLM) were fitted to the data to test the hypotheses. All analyses were computed using the statistical environment R (R Core Team, 2018) using the "nlme" package for MLM (Pinheiro et al., 2019) with a restricted maximum likelihood method of estimation. Intercepts on each level were always set at random to account for potential bias on standard errors due to the nested structure of the data. Centering of predictor variables was performed according to standard procedures for MLM (Brincks et al., 2017). Random effects were only fitted when they improved the model fit. To find the best-fitting model, different models were compared using the Akaike information criterion (AIC) as well as likelihood ratio test for nested models. Each final model was tested for violations of central model assumptions. As sensitivity analyses, we tested whether: (a) home practice, feasibility of the stress management exercises, and perceived benefits of the intervention (all retrospectively assessed) were associated with changes in the outcome variables (AUCs, stress, mindfulness) within the IG and whether (b) prior experiences with yoga, relaxation techniques or any other MBI would change the results of the main models. Since there were no relevant associations and since the results of the models remained unchanged, we do not report on results from these sensitivity analyses. Furthermore, only results necessary to investigate the hypotheses of interest (focal predictors) are reported.

2.4.1 | Models testing for intervention effects

Two-level MLM's were built with pre-post measurements at level 1, nested in individuals at level 2. To represent pre- and post-measurement values, AUCg, AUCi, mindfulness, and perceived stress from days 1 and 2 were averaged to create the pre-intervention measurement, while average values from days 3 and 4 constituted the post-intervention measurement. Likewise, several predictor variables (covariates) from the EMA assessment were averaged for pre- and post-intervention and centered around their person mean (minutes after awakening, sleep quality, eating, drinking, caffeine intake, and physical activity). BMI and age (both grand-mean centered) as well as sex, smoking, hormonal contraceptive intake, and menstrual cycle phase (all dummy coded) were entered as additional covariates.

All averaged AUCg's and AUCi's were screened for validity. In this process, two averaged sAA AUCi extreme values (4.88 and 5.08 *SD* below the mean) were considered invalid and, thus, excluded from the dataset before analysis. Given that the averaged sAA AUCg's, sAA AUCi's, and sCort AUCg's were positively skewed, they were transformed to the natural logarithm to enable an approximate normality of the MLM residuals. Lastly, measurements beyond 3 *SD* of the mean were considered as outliers and excluded.

Based on the same procedures, the effects of the MBI on pre-post changes in averaged subjective measures (perceived mindfulness and stress) were calculated controlling for sex and age. Averaged perceived mindfulness levels were transformed using the box cox transformation to enable an approximate normality of the MLM residuals.

A group-by-time interaction was used as a focal predictor in all models to predict changes in the parameters of interest.

2.4.2 | Models testing for the momentary association of mindfulness, alpha-amylase, cortisol, and stress

Three-level MLM's were fitted to predict sAA, sCort and perceived stress at each measurement occasion (on level 1 nested in days on level 2 and in individuals on level 3). Cortisol and sAA were transformed using the natural logarithm to enable an approximate normality of the MLM residuals. Thereafter, measurements beyond 3 *SD* of the mean were excluded from the analyses. To account for the circadian rhythm of sAA and sCort, the time of assessment relative to awakening was controlled in both models. Exploratory analyses indicated a quadratic trend of time for sAA and sCort within days. Hence, it was considered as covariate. In a next step, physical activity as well as food, caffeine, and drink consumption were centered on their daily average. Additional covariates were either dummy coded (smoking, sex, menstrual cycle phase and hormonal contraceptive intake), centered on their person mean (minutes after awakening) or on the grand mean (age and BMI). To control for a possible effect of the intervention on the associations of interest, a group-by-time interaction was considered as additional covariate. In the process of model building, random slopes for the linear and quadratic trend of time on level 3 were found to improve the model fit. Furthermore, the addition of a continuous autocorrelation structure for the within-subject residuals on level 1 as a function of time between adjacent measurements improved the model fit for the sCort- but not for the sAA-model.

The three-level MLM to predict perceived stress in everyday life was fitted based on the same deliberations and procedures as the other two models. However, in this model we only considered a linear trend of time as well as sex, age, and the group-by-time interaction as covariates. An improvement in model fit was found by allowing random slopes for the linear trend of time on level 2 and level 3 as well as by adding a continuous autocorrelation structure as described above.

Momentary assessments of mindfulness were centered on their daily average. This variable was then used as focal predictor to predict momentary fluctuations of the three parameters of interest.

3 | RESULTS

3.1 | Sample characteristics and descriptive data

Baseline values of all variables were compared between groups (Table 1), indicating marginal differences only for BMI.

3.2 | Intervention effects

3.2.1 | Alpha-amylase

The first model, predicting averaged sAA AUCg based on the participation in the intervention showed a statistically significant group-by-time interaction ($b = -0.361$, $p = .043$), as depicted in Figure 1, where participants from the IG showed a decrease in sAA levels over time (change score $M = -6244.69$, $SD = 44{,}386.82$), while the opposite was the case for the CG (change score $M = 9{,}051.98$, $SD = 45{,}935.82$). There was no effect of the intervention on the sAA AUCi ($b = 0.012$, $p = .798$).

3.2.2 | Cortisol

The decrease of averaged sCort AUCi levels was not significantly different between the IG and the CG ($b = -84.414$, $p = .093$). However, descriptively, stronger change scores were observed in the IG ($M = -97.56$, $SD = 211.04$), as compared to the CG ($M = 12.76$, $SD = 202.53$). There was no effect of the intervention on sCort AUCg ($b = -0.025$, $p = .800$).

3.2.3 | Self-report measures

Average mindfulness and average perceived stress-levels showed no significant group-by-time interactions (both $p \geq .592$).

3.3 | Association of momentary mindfulness with alpha-amylase, cortisol and perceived stress

Momentary state mindfulness was negatively associated with momentary-levels of sAA ($b = -0.087$, $p = .012$) and momentary perceived stress ($b = -5.3403$, $p < .000$), but not with momentary secretion of cortisol ($b = 0.014$, $p = .523$).

4 | DISCUSSION

No study to date investigated the effects of MBI on everyday life stress, as captured through day-to-day and moment-to moment assessments of state mindfulness, perceived stress, and stress-sensitive psychobiological markers of the SNS (sAA) as well as of the HPA-axis (sCort).

We found that an MBI reduced aggregated sAA (AUCg), and – although with a smaller effect – improved the natural sCort decline throughout the day (AUCi). Thus, we were able to show that sAA, a sensitive SNS outcome, can be downregulated by an MBI, as compared to a control condition, in the context of increasing stress over a period of three months caused by a major exam which all participants had to face at the end of the semester. This is in line with our hypotheses and supports the notion that MBI can improve indicators of mental and physical health. Specifically, research on the neural mechanisms underlying MBI suggests that MBI can reduce SNS activity via increased prefrontal (top down) regulation of stress-related amygdala activation (Weinstein et al., 2009), and reduced catecholamine-secretion (Creswell & Lindsay, 2014), which would then lead to reduced sAA. In contrast, we found no effects of the MBI on subjective stress or everyday mindfulness, which might be caused by the fact that the two day-EMA at post-intervention was assessed only a few days before the exam, possibly leading to high levels of psychological strain.

While the results regarding the MBI effects on psychological and biological parameters were dissociated when using simple pre-post-averages, we found associations among those parameters on a moment-to-moment level. More specifically, increased mindfulness was associated with reduced sAA concentrations and perceived stress, however not with momentary sCort. The current state of studies seems to indicate that at the moment-to-moment level mindful awareness is accompanied by an immediate reduction in autonomic nervous-system activation (Creswell & Lindsay, 2014; Nyklicek et al., 2013), which as a consequence would result in lower momentary sAA levels. In contrast, the HPA axis reacts much slower, with stress-associated sCort increases about 20 min after a stressful situation. With such a delayed response-pattern, an immediate association of mindfulness and sCort can only be expected with a more stable state of mind—which then, therefore, shows only limited momentary variability.

Importantly, the EMA approach we used avoids error-prone retrospective data collection (Robinson & Clore, 2002) and enables reliable investigations of the temporal changes at a high resolution which allows for the analysis of within and between-subject effects.

TABLE 1 Sample characteristics and descriptive data at pre- and post-measurement

	Pre		Post		Group comparisons[a]		
	IG ($n = 28$)	CG ($n = 46$)	IG ($n = 27$)	CG ($n = 45$)	χ^2 -Statistics	*df*	*p*-value
Sex (female)	$n = 18$ (64.3%)	$n = 30$ (65.2%)	$n = 18$ (66.7%)	$n = 29$ (64.44%)	0	1	1.000
Smokers	$n = 7$ (25%)	$n = 7$ (15.2%)	$n = 5$ (18.5%)	$n = 4$ (8.9%)	0.542	1	.462
Hormonal contraception (yes)	$n = 9$ (32.1%)	$n = 19$ (41.3%)	$n = 11$ (40.7%)	$n = 17$ (37.8%)	0.293	1	.589
Follicular phase (yes)	$n = 9$ (32.1%)	$n = 16$ (34.8%)	$n = 10$ (37%)	$n = 13$ (28.9%)	0	1	1
Lutealphase (yes)	$n = 5$ (17.9%)	$n = 6$ (13%)	$n = 5$ (18.5%)	$n = 9$ (20%)	0.064	1	0.8
Intake of caffeine[b]	$n = 58$ (17.9%)	$n = 66$ (12%)	$n = 68$ (21.4%)	$n = 74$ (13.9%)	5.627	1	.018

	IG Mean (*SD*)	IG Min, Max (Range)	CG Mean (*SD*)	CG Min, Max (Range)	IG Mean (*SD*)	IG Min, Max (Range)	CG Mean (*SD*)	CG Min, Max (Range)	*t*-Statistics	*df*	*p*-value
Age	20.79 (1.57)	19.00, 24.00 (5.00)	21.41 (3.69)	18, 35 (17.00)	21.33 (1.64)	19.00, 25.00 (6.00)	21.40 (3.25)	18.00, 36.00 (18.00)	1.013	66.081	.315
Body-mass-index	20.85 (2.05)	17.51, 26.30 (8.79)	22.62 (3.05)	14.88, 31.74 (16.86)	21.00 (1.98)	17.21, 26.01 (8.81)	22.43 (2.82)	15.24, 30.47 (15.23)	2.986	71.181	.004
Minutes since wake-up[c]	8.14 (5.89)	1.50, 25.00 (23.50)	6.59 (7.78)	0.50, 43.50 (43.00)	5.04 (3.55)	1.00, 15.00 (14.00)	5.36 (4.60)	0.00, 20.00 (20.00)	−0.973	69.474	.334
Sleep quality[c]	60.14 (17.60)	16.00, 86.50 (70.50)	63.38 (20.71)	27.50, 100.00 (72.50)	58.48 (17.68)	16.00, 98.00 (82.00)	65.52 (16.58)	25.50, 100.00 (74.50)	0.717	64.28	.476
Intake of meal[c]	30.36 (7.81)	11.56, 45.70 (34.14)	32.89 (8.86)	16.38, 56.44 (40.07)	30.93 (6.64)	16.56, 45.25 (28.69)	31.27 (7.71)	15.00, 48.00 (33.00)	1.286	62.766	.203
Intake of drink[c]	32.14 (8.95)	16.56, 50.10 (33.54)	32.33 (9.05)	14.30, 56.10 (41.80)	32.05 (7.49)	19.20, 56.30 (37.10)	29.35 (8.87)	11.30, 52.80 (41.50)	0.086	57.626	.932
Day activity[c]	31.46 (10.32)	9.75, 51.00 (41.25)	28.86 (8.85)	10.60, 45.20 (34.60)	26.80 (7.66)	10.00, 40.22 (30.22)	24.86 (8.74)	12.00, 48.90 (36.90)	−1.108	50.538	.273
Cigarettes smoked per day[c]	2.9 (0.96)	1.50, 4.00 (2.50)	5.14 (6.05)	1.00, 18.00 (17.00)	2.00 (1.27)	1.00, 4.00 (3.00)	7.25 (8.51)	2.50, 20.00 (17.50)	0.965	6.421	.370
sAA AUCg[c]	58,056.42 (42,580.34)	12,559.51, 180,101.64 (167,542.14)	53,187.00 (45,645.16)	8,037.60, 255,117.98 (247,080.38)	52,931.27 (55,303.40)	8,347.10, 295,645.22 (287,298.13)	61,689.87 (60,977.57)	2,851.58, 328,260.10 (325,408.51)	−0.449	55.879	.656
sCort AUCg[c]	206.30 (72.79)	74.78, 380.56 (305.78)	217.33 (72.20)	98.03, 425.15 (327.11)	190.60 (72.48)	87.01, 341.09 (254.07)	197.33 (62.80)	74.27, 362.34 (288.07)	0.620	51.661	.538
sAA AUCi[c]	19,353.23 (41,564.27)	−101371.24, 90,135.81 (191,507.06)	13,689.24 (46,257.78)	−149172.83, 116,113.41 (265,286.25)	8,776.20 (65,149.23)	−152322.97, 218,426.36 (370,749.33)	2,804.32 (77,480.03)	−277240.33, 206,277.86 (483,518.19)	−0.525	57.328	.601
sCort AUCi[c]	−158.88 (146.44)	−469.56, 132.04 (601.60)	−171.21 (159.66)	−549.06, 84.61 (633.67)	−245.69 (156.13)	−622.72, −8.96 (613.76)	−159.34 (141.70)	−473.92, 96.93 (570.85)	−0.332	55.868	.741
State mindfulness[c]	5.63 (0.70)	4.33, 6.92 (2.58)	5.88 (0.79)	3.68, 6.95 (3.26)	5.69 (0.85)	4.00, 6.98 (2.98)	5.88 (0.86)	3.62, 7.00 (3.38)	1.415	62.971	.162
Stress[c]	33.30 (12.84)	3.17, 50.08 (46.92)	30.83 (12.42)	5.27, 53.67 (48.39)	37.46 (12.65)	9.60, 68.83 (59.23)	34.57 (13.28)	5.17, 56.75 (51.58)	−0.812	55.674	.421

Note: Sex: 0 = man, 1 = woman; Caffeine = yes/no; Smoker = yes/no; hormonal contraception = yes/no; menstrual cycle phase = two variables: (1) luteal (yes/no), follicular (yes/no). Intake of caffein (0 = no, 1 = yes): *Have you consumed caffeine since the last saliva sample?;* BMI: kg/m^2; sleep quality: *how well did you sleep* (visual analog scale from 1 = very poor to 100 = very good); intake of meal: *Have you eaten anything since the last saliva sample?* (visual analog scale: 1 = small amount to 100 = large amount); intake of drink: *Have you drunk anything since the last saliva sample?* (visual analog scale: 1 = small amount to 100 = large amount); day activity: *How physically active have you been since the last saliva sample?* (visual analog scale: 1 = little activity to 100 = very active).

[a]Where applicable, *p*-values were obtained from significance tests conducted to compare baseline characteristics between groups (i.e., pre-measurments). χ^2- tests were used in case of categorical variables. Welch's *t* tests were used to compare continuous variables .

[b]Number of occassions at which participants reported to have consumed caffeine since the last prompt.

[c]These mean values are based on mean values of day 1 and 2 of the pre-measurement point (baseline).

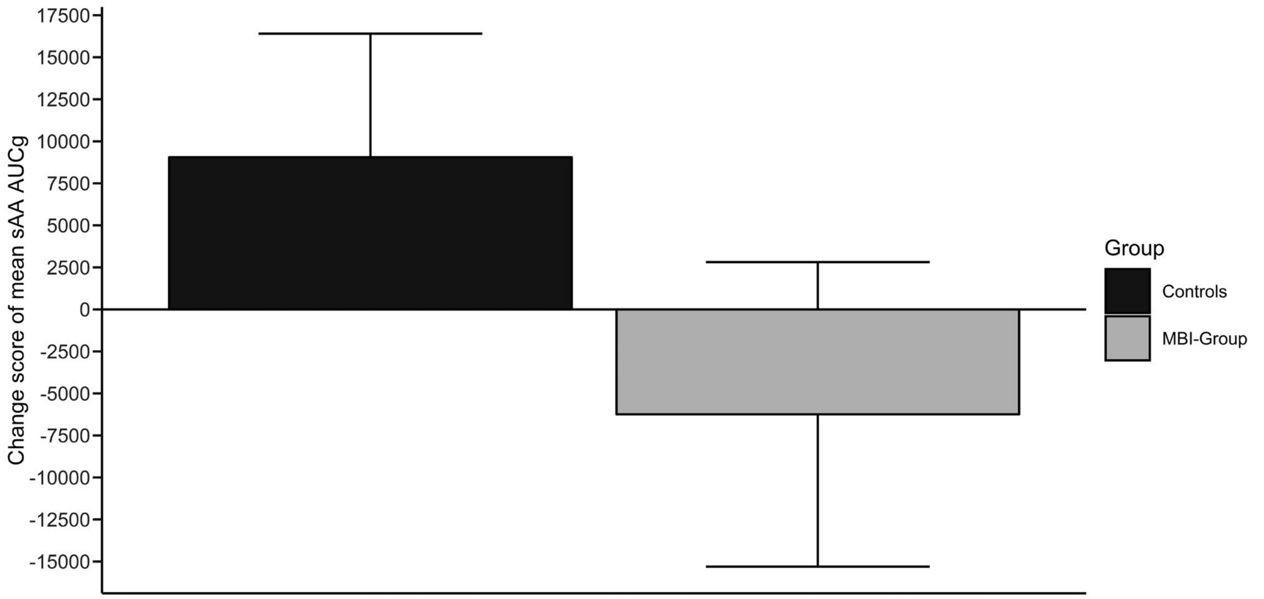

FIGURE 1 Model predicting total saliva alpha-amylase output based on the participation in the intervention: Group-by-time interaction effect

As described above, our results indicate that it is very beneficial to separate different levels of aggregation. Furthermore, unlike EMA-based approaches with a high ecological validity, measurements under laboratory conditions can lead to different and possibly unexpected results. In a recent study, for example, an inverse relationship was found between mindful awareness and parasympathetic activation, which the authors believe to be linked to laboratory-related aspects such as higher vigilance, higher cognitive load, etc. (Watford et al., 2020).

In summary, our findings are in line with previous studies suggesting that MBI can improve stress-associated health indicators (Creswell et al., 2014; Heckenberg et al., 2018; Pascoe, Thompson, Jenkins, et al., 2017; Pascoe et al., 2017; Sanada et al., 2016), especially in medical students, who are particularly vulnerable to stress-related disorders (Daya & Hearn, 2018; Rotenstein et al., 2016), and expand the findings to the context where they matter—in everyday life. However, our data also suggest that MBI does not reduce subjective stress or sCort, but average sAA. Furthermore, our research adds an entirely new layer of information by providing the first proof-of-concept indication that a momentary state of being mindful in fact predicts concurrently lower SNS activity and lower levels of subjective stress.

Our results emphasize the importance of momentary mindfulness and the associated emotion regulation strategy—especially the acceptance contained therein—for coping with stress in everyday life (Lindsay et al., 2018). The significance for health is additionally underpinned by the psychophysiological altered correlates associated with it. This implies that brief interventions for example, for integration into everyday life have been underestimated in psychobiological intervention research so far. Future studies should investigate this aspect in greater depth in order to support the few established findings on this topic.

5 | LIMITATIONS

In order to control for external factors and have a valid stressor for all participants, we investigated a highly selective and overall healthy convenience sample, with an unequal distribution with considerably more female medical students. This limits the generalizability of our results, especially as women and men respond very differently to stress.

Whether participants chose the MBI or another course as part of their medical curriculum was controlled but not randomized in this study. Given the near exam for all participating students, neither randomization nor a waitlist-control condition seemed feasible or ethically justified. Thus, although we did not find baseline differences in subjective stress, mindfulness, sAA, or sCort between groups, other factors might have differed which could have moderated our results.

6 | CONCLUSIONS

Our findings suggest that MBI can reduce psychobiological stress markers, assessed via repeated measures of sympathetic and HPA-axis outcomes in everyday life. In addition, state mindfulness is associated with the lowering of sAA secretion and perceived stress on a momentary basis. This underlines how with voluntary and cognitive mechanisms, healthy individuals can learn to down-regulate autonomic arousal and subjective stress during phases of high demand. The data, thereby, expand on the current literature and suggest that the positive effects of mindfulness-based techniques are not only restricted to the general before-after rationale, but are also reflected in everyday life. Targeted momentary interventions might build upon these effects and be used to improve not only subjective but also health-related physiological outcomes in daily life.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Writing-original draft; Writing-review & editing: Corina Aguilar-Raab. *Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Visualization; Writing-original draft; Writing-review & editing*: Martin Stoffel. *Formal analysis; Writing-original draft*: Cristóbal Hernández. *Data curation*: Stefanie Rahn. *Methodology; Resources; Software*: Markus Moessner. *Methodology*: Barbara Steinhilber. *Conceptualization; Funding acquisition; Investigation; Resources; Supervision*: Beate Ditzen.

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