Disaggregating between- and within-person relations for depression and burnout symptoms with cumulative hair cortisol and endocannabinoid concentrations: A multilevel analysis of four annual assessment waves

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Abstract

Background: The endocannabinoid system (ECS) is increasingly being recognized as key regulatory system coupled with the glucocorticoid system implicated in the pathophysiology of stress-related mental disorders. However, prior studies examining the ECS in depression or burnout have been inconclusive, of small sample size or of cross-sectional nature limiting interpretations of causal inferences or time-dependent effects.

Methods: In a prospective community based cohort study including 128 individuals (women: 108), depression (PHQ-9) and burnout symptoms (MBI-GS) as well as hair cortisol and endocannabinoids were measured annually over four years. Cortisol, arachidonylethanolamide (AEA) and 2-arachidonyl-sn-glycerol/1-arachidonyl-sn-glycerol (2-AG/1-AG) were extracted from 3 cm hair segments reflecting cumulative concentrations of the last 3 months prior hair sampling.

Results: Cross sectional group comparisons at baseline revealed reduced cortisol and AEA levels in the group with a positive major depressive disorder screening compared to individuals with low depression symptomatology (both $p < .05$). Cortisol was also reduced in the group with a positive burnout screening compared to individuals with a negative screening at baseline ($p < .05$). Longitudinal multilevel analysis, showed for the total sample that a within-person increase in burnout symptoms was associated with a decrease in cortisol levels over time ($p < .05$). In the male subsample, a between-person increase in AEA levels across time was associated with a decrease in depression symptoms and vice versa. However, a within-person increase of AEA levels across time was associated with an increase in depression symptoms and vice versa (both $p < .05$). Further, a within-person increase in burnout symptoms lead to a significant increase in AEA levels in the male subsample ($p < .05$).

Conclusions: While cross-sectional analyses suggest higher depression or burnout symptomatology to be associated with reduced cortisol and AEA release, longitudinal analyses disaggregating between- and within-person effects reveal a complex pattern. A within-person increase in burnout symptoms precedes a decrease in cortisol secretion suggesting an exhaustion of the HPA axis. Between-person analysis show only for men a negative association between depression symptoms and AEA levels over time. However, within-person effects, indicate an increase in depression symptoms to precede and to follow an increase in AEA levels, suggesting a dynamic counterregulatory mechanism between the EC and depression in men differing on the between- and within-person levels. These longitudinal associations further

elucidate the time and sex dependent relation between depression, burnout, glucocorticoid and endocannabinoid secretion.

Introduction

Depressive disorders are considered stress-related mental disorders characterized by its core symptoms of depressive mood and anhedonia (Smoller, 2015). Similarly, also the burnout syndrome is considered a stress-related psychological state identified by emotional exhaustion, reduced professional efficacy, and cynicism caused by an excess of work-related stress (Schaufeli et al., 2009). Although, the underlying pathophysiology of depressive disorders and burnout are insufficiently understood, a dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis has been identified in both conditions in many independent investigations (Otte et al., 2016; Rothe et al., 2020).

However, the endocannabinoid system (ECS) is increasingly being recognized for its stress-responsive and -regulatory properties functionally interacting with the HPA axis (Balsevich et al., 2017). That is, glucocorticoids such as cortisol – the main effector of the HPA axis – recruit endocannabinoid signaling and thereby the ECS modulates many of the neurobiological effects of glucocorticoids (Balsevich et al., 2017). The HPA axis together with the ECS are therefore regarded as two interlocking key regulatory systems implicated in the pathophysiology of depressive disorders (Hillard, 2017) and burnout (Gao et al., 2020). This perspective is substantiated by the fact that both, glucocorticoid receptors (GRs) as well as cannabinoid receptors (CBR) are expressed in all of the main brain structures involved in depressive disorders and burnout such as the hypothalamus, hippocampus, habenula, cortex, limbic system and amygdala (Biselli et al., 2019; Gururajan et al., 2019).

Although it seemed widely accepted that major depressive disorder (MDD) is associated with increased HPA activity and increased basal cortisol concentrations (Otte et al., 2016; Stetler and Miller, 2011), this is no longer unambiguous given reduced cortisol stress reactivity in MDD (Miller and Kirschbaum, 2019; Rothe et al., 2020) and the integration of new specimen such as hair cortisol showing mixed associations (Behnke et al., 2021; Gerritsen et al., 2019; Rothe et al., 2021, 2020; Stalder et al., 2017a; Steudte-schmiedgen et al., 2017). With regard to the ECS, on the one hand, rodent studies using cannabinoid type 1 receptor (CB₁R) knock-out models, pharmacological blockade or stimulation indicate increased signaling of the CB₁R in the central nervous system to exert antidepressant and anxiolytic

effects (Aso et al., 2008; Bambico et al., 2007; Gobbi et al., 2005; Haller et al., 2001; Kathuria et al., 2003), while on the other hand, there is a report of impaired CB₁R signaling by pharmacological blockade being associated with antidepressant effects but increased HPA activation (Steiner et al., 2008). By contrast, chronic unpredictable stress significantly increasing HPA activity was shown to reduce the endocannabinoid N-arachidonylethanolamine (anandamide [AEA]) in the central nervous system (Hill et al., 2008), while concomitantly decreasing CB₁R binding site density in the hippocampus and hypothalamus, but increasing it in the prefrontal cortex. Examining acute and chronic corticosterone administration further substantiated that the CB₁R is under negative regulation by glucocorticoids (Hill et al., 2008a). However, examining specific brain regions such as the lateral habenula, increased levels of 2-arachidonoylglycerol (2-AG) are reported after chronic unpredictable or social defeat stress, while site specific CB₁R blockade reduced anxiety behavior and increased basal corticosterone (Berger et al., 2018). Compelling evidence for an antidepressive effect of CB₁R activation in the amygdala^{CK}-nucleus accumbens^{D2} circuit was recently provided (Shen et al., 2019), suggesting functionality of the CB₁R and the availability of activating compounds such as AEA and 2-AG to be crucial for preventing the establishment of depressive disorders.

Human studies including patients suffering from major depressive disorder (Hill et al., 2009; Hill et al., 2008b), or post-traumatic stress disorder (PTSD) show on the one hand reduced serum AEA and 2-AG levels (Hill et al., 2013; Neumeister et al., 2013), but on the other hand no associations or increased levels of AEA and 2-AG are also reported for these disorders or related conditions (Giuffrida et al., 2004; Harfmann et al., 2020; Hauer et al., 2013; Schaefer et al., 2014). However, experimental research showed that under stress, the enzyme fatty acid amide hydrolase (FAAH), which downregulates AEA and to a lesser extent 2-AG, is activated in the amygdala (Hermanson et al., 2013). Inhibition of FAAH and thereby an increase in AEA emerges as protective treatment against the anxiogenic effects of stress in healthy individuals (Mayo et al., 2020). Another study showed that chronic stress (520 days of isolation and confinement) reduced 2-AG, but not AEA levels in healthy men (Yi et al., 2016). A recent study further highlights that inflammation-induced depression is not associated with circulating AEA or 2-AG levels suggesting alterations in endocannabinoid levels in depression to be unrelated to inflammatory-dependent depression states (Zajkowska et al., 2020).

While serum endocannabinoid levels or levels extracted from the central nervous system vary with respect to the acute situation or time of the day, concentrations obtained from hair – suggesting to be long-term integrated concentrations over several months – are not subject to many of these confounding variables. A study revealed reduced hair AEA, but not hair 2-AG, levels in patients with borderline personality disorder compared to healthy controls, while a negative correlation between depressive symptoms and hair AEA levels was observed (Wingenfeld et al., 2018). Another study further reported negative correlations between hair endocannabinoid levels of N-acyl-ethanolamides palmitoylethanolamide (PEA), oleoylethanolamide (OEA), stearoylethanolamide (SEA) and PTSD symptom severity (Wilker et al., 2016). However, another report examining a sample of male refugee minors in a cross-sectional design did not identify consistent associations between psychological symptomatology and hair endocannabinoids except for a positive correlation between 2-AG/1-AG and depressive symptoms (Croissant et al., 2020). Related to this line of research is a study suggesting higher hair 1-AG and lower SEA levels in women with childhood maltreatment (Koenig et al., 2018). In a recent analysis, we further replicated significant negative cross-sectional relations between hair AEA levels and anxiety and burnout, and on trend-level with depression symptoms in a sample of 207 individuals of the general population (Gao et al., 2020). However, a recent analysis comparing 21 depressed women with 27 nondepressed controls identified no differences between groups for hair endocannabinoids (Behnke et al., 2021).

Taken together, based on rodent studies and cross-sectional human studies a state of endocannabinoid deficiency is suggested to represent a stress-susceptible endophenotype predisposing to depression or other stress-related mental conditions (Giacobbe et al., 2021; Gorzalka and Hill, 2011; Hill et al., 2018). However, although there is compelling evidence that endocannabinoid levels and CB₁ signaling are reduced in depression or other stress-related mental conditions, longitudinal human studies dissecting between- and within-person effects by examining alterations in long-term integrated endocannabinoid and glucocorticoid levels in parallel with regard to changes in depression or burnout symptoms are lacking.

Methods and Materials

Sample characteristics and study procedure

Participants were members of the Dresden Burnout Study, a prospective cohort study, which tracks changes in psychological symptoms and corresponding changes in different biological systems related to mental health approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki (Penz et al., 2018a). Recruitment of participants was achieved via a local population registry sending invitation letters of the study to inhabitants of the city of Dresden meeting age inclusion criteria selected by random sampling. Furthermore, public media platforms, newspaper announcements and radio broadcasts advertised the study and invited to participation.

Participants had to register for the study via the Dresden Burnout Study - Online Platform and were subsequently selected and invited for biomarker testing when living in the nearer area of Dresden. One week prior to their examination participants were invited via email to complete an online psychometric test battery including depression and anxiety measures. At the day of the biological examination, participants arrived overnight fasting and responded to a standardized set of questions providing information on the current constitution. Subsequently, several biological measures were obtained including blood serum and hair sampling as described in more detail in the official study protocol (Penz et al., 2018a). Should the participants not have completed the online questionnaire battery by the date of examination, they would complete the questionnaire after the biological examination on site. For the purpose of this longitudinal examination, only participants who participated in at least three out of four examinations of the annual biomarker survey during the period between 2015 and 2018 were included.

Psychometric evaluation by self-report questionnaires

Depressive symptoms were measured using the Patient Health Questionnaire-9 (PHQ-9; Kroenke et al., 2001). The PHQ-9 comprises 9 items reflecting the DSM-IV criteria for a major depressive disorder (e.g. feeling down, depressed, or hopeless). Assessing how often the respondent has been bothered by specific symptoms over the past two weeks using a four-point Likert scale of 0 to 3 points (0 - not at all, 3 - nearly every day), the PHQ-9 ranges between 0 and 27. Large validation studies examining PHQ-9 cut-off scores (e.g. ≥ 10) compared to structured clinical interviews confirm good detection performance of depressive disorders indicating individuals with scores of 10 or higher to have high depressive burden and to be

consistently identified as having MDD by clinical diagnostic interviews (Levis et al., 2020). A Cronbach's alpha of .88 emerged for this sample suggesting a good internal consistency.

Burnout symptomatology was measured using the Maslach Burnout Inventory-General Survey (MBI-GS; Büssing and Glaser, 1999; Schutte et al., 2000), which is considered the gold standard for assessing burnout by self-report. The MBI-GS comprises of 16 items rated on a seven-point Likert scale (0 - never, 6 - daily) examining symptoms experienced in relation to one's work. Three subscales representing the burnout dimensions emotional exhaustion, professional inefficacy, and cynicism can be derived, while the MBI-GS weighted sum score ranges between 0 and 6. As cumulative cut off value indicating a clinically relevant form of burnout symptomatology, a summed value of 3.5 or higher was suggested (Kalimo et al., 2003). A Cronbach's alpha of .92 emerged for the total score for this sample suggesting a good internal consistency.

Endocannabinoid and glucocorticoid quantification

For the quantification of endocannabinoids and glucocorticoids, three hair strands (each with a minimum of 20 mg of hair) were cut as close as possible to the scalp from the posterior vertex. Hair samples were processed and analyzed as described in our published protocols for glucocorticoids (Gao et al., 2013) and endocannabinoids (Gao et al., 2020). Sample pre-processing and subsequent biochemical analysis were conducted at the Dresden LabService GmbH (Tatzberg 47, 01307, Dresden, Germany). Samples were cut into 3cm segments from the scalp-near site representing the integrated hormone concentration over the last three months (e.g. average hair growth rate 1cm per month; Wennig 2000) and weighted into 7.5 mg of whole, nonpulverized hair and subsequently washed with 2.5 ml isopropanol following the protocol by Gao et al. (2020, 2013). Biochemical analysis was conducted using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) described in detail elsewhere (Gao et al., 2016). To avoid batch effects, all samples of the 128 participants were quantified in a single run after the study was completed end of 2018. The period between hair sampling and biochemical analysis varies therefore for the samples between 0 to 4 years, depending on the study visit of the participants and the obtainment of the sample. This variable was further included as covariate in the analysis. However, storage duration effects have so far not been reported, while batch effects pose a well-recognized issue in biochemical research across the globe. Recently, strong test-retest associations of hair endocannabinoid

levels were reported for the used method with intraclass correlation coefficients between 0.80 and 0.92 were reported (Gao et al., 2021). Intra- and inter-assay coefficient of variation for endocannabinoids are below 14% suggesting acceptable quantification performance (CV < 15 %) (Caruso et al., 2008), and for cortisol and cortisone are below 8.8 % and 8.5 %, respectively. The lower limit of detection for endocannabinoids is below (or equal to) 0.3 pg/mg⁻¹ for AEA, 2-AG/1-AG, and 6 pg/mg⁻¹ for PEA, OEA, SEA, while for cortisol and cortisone it is 0.09 pg/mg and 0.07 pg/mg.

Statistical analysis

Cortisol and endocannabinoid data was log-transformed in order to approach a normal distribution. Level of significance was set at $p < .05$. For calculation of means, standard deviations (SD) and cross-sectional comparisons, the first timepoint was used. Two-tailed t-tests were conducted in order to perform cross-sectional comparisons between men and women with regard to general demographics, endocannabinoids and clinical parameters (depression and burnout).

Two-tailed t-tests were also used in order to conduct cross-sectional comparisons with regard to severity of psychological disorders, cortisol and endocannabinoids. Based on the PHQ-9 screening measure a cut off of ≥ 10 was used in order to create a major depression syndrome subsample (Levis et al., 2020). For the MBI-GS scale a cut-off of ≥ 3.5 was used in order to generate a subsample consisting of individuals suffering from burnout (Kalimo et al., 2003). Due to the small portion of men in the present sample, we did not conduct subsample analyses based on sex with regard clinical parameters.

Based on the nested structure of the present data, multilevel modelling was used (Raudenbush and Bryk, 2002). The data had a two-level structure, meaning that years were nested within individuals. Multilevel regression analyses were used to examine whether cortisol and endocannabinoid levels predicted clinical parameters and vice versa. The models were estimated with the lme4 package (Bates et al., 2015) in RStudio (version 3.3.3, (R Core Team, 2017)). Models were fitted with maximum likelihood estimation. Model comparisons were calculated with the anova function of the car package (Fox et al., 2007) and revealed that a random-intercept model provided the best fit for the present dataset. 500 simulations were used in order to compute parametric bootstrap intervals with the confint.merMod

function from the lme4 package (Bates et al., 2015). The lme4 package does not provide p-values due to estimation of degrees of freedom and denominator degrees due to disagreements in the literature (Bates et al., 2015). The Satterthwaite's degree of freedom approach (Satterthwaite, 1941) and the Kenward-Roger approximation (Kenward and Roger, 1997) represent methods which produce the most acceptable Type I error rate, even for small sample size (Luke, 2017). We applied the Satterthwaite approximation as it can be applied to models fitted with a maximum likelihood approach, whereas the Kenward-Rogers approximation only applies to REML models (Luke, 2017).

All predictors were person-mean centered to capture within-person variations of endocannabinoids and clinical parameters. Additionally, to control for between-person effects, endocannabinoids, depression and burnout were grand-mean centered and included into the respective models (Hoffman and Stawski, 2009). Furthermore, in our analyses we controlled for age, sex, BMI, medication intake, and alcohol consumption (Best et al., 2021; Stalder et al., 2017a, 2010). We conducted subsample analyses for both sexes.

Results

Descriptive Statistics

Descriptive statistics separated by sex are summarized in Table 1. The total sample consisted of 128 participants, with a mean age of 44.96 years, 84.38% of the overall sample was female. In total 30 participants (23.4%) were identified as suffering from a major depressive syndrome based on the cut-off point of the PHQ-9 ≥ 10 , while 19 participants (14.8%) reached the cut-off point for a burnout syndrome of the MBI ≥ 3.5 . A mean BMI of 25.8 was observed and 15.6% self-identified as regular tobacco smokers and 19.5% reported to drink alcohol once in a week or more often. A total of 32.8% reported to suffer from a chronic disease and the same percentage reported to use medication due to their condition, while 14.8% reported to use multiple medications. We used unpaired two-sided t-tests and χ^2 tests to assess sex differences. Men and women did not significantly differ with regard to age, BMI, chronic disease status, alcohol consumption, smoking status, medication intake, mental disorder status, burnout, or major depressive syndrome. As shown in the Supplementary Figures 1 and 2, there were no clear sex differences in depression symptoms and burnout symptoms, cortisol, AEA, 2-AG/1-AG, OEA, PEA and SEA levels. Baseline distribution of the parameters separated by sex are provided in Supplementary Figures 3 (for pg/mg values) and 4 (for log

values). Intraclass correlation coefficients (ICCs) for depressive symptoms from T1 to T4 were .76 for the overall sample, .72 for women and .87 for men. ICCs concerning burnout symptoms were .66 for the overall sample, .62 for women and .85 for men, respectively. ICCs concerning cortisol and endocannabinoids levels were .25 - .39 for the overall sample, .20 - .42 for women and .24 - .53 for men.

Cross-sectional baseline comparisons of endocannabinoid- and cortisol levels by clinical features

As presented in Figure 1, significant differences emerged between the major depressive syndrome group (based on the PHQ-9 screening measure cut off ≥ 10) and participants with low depressive symptoms concerning cortisol ($t = -2.554, p < .05$) and AEA ($t = -2.053, p < .05$). Individuals in the major depressive syndrome group exhibited significantly lower cortisol ($M = .550, SD = .339$) and AEA levels ($M = -.483, SD = .287$) whereas the group with low depressive symptomatology had significantly higher cortisol ($M = .804, SD = .370$) and AEA levels ($M = -.225, SD = .452$). No differences were found for 2-AG/1-AG, OEA, PEA and SEA (all $p > .05$).

As shown in Figure 2, individuals with burnout (based on the MBI-GS screening measure cut off ≥ 3.5) significantly differed from individuals with low burnout symptoms concerning cortisol levels ($t = -3.160, p < .01$). Participants with burnout had significantly lower cortisol levels ($M = .458, SD = .289$) compared to individuals who had low burnout symptoms ($M = .798, SD = .367$). No significant differences were found between the two groups with respect to AEA, 2-AG/1-AG, OEA, PEA and SEA (all $p > .05$). Figures 3 and 4 present longitudinal change patterns for the six different analytes separated by syndrome group at baseline indicating no consistent differences between the groups across time (data not shown). Therefore, multilevel regression analysis disaggregating between- and within person associations shall be described in more detail in the following.

Multilevel regression

Depressive symptoms (PHQ-9), endocannabinoids and cortisol

In the male subsample, a within-person increase of AEA significantly predicted an increase in symptoms of depression across time ($b = 27.504, p < .05, 95\% \text{ CI } [10.324; 44.460]$) (see table 2). A within-person increase of PHQ-9 scores did significantly predict an increase in AEA levels ($b = .061, p < .001, 95\% \text{ CI } [.020; .103]$) (see table 3). The direction of the slope in question was reversed when looking at between person differences of the male subsample, an increase in

AEA significantly predicted a decrease in PHQ-9 levels ($b = -24.389$, $p < .05$, 95% CI [-41.138; -7.150]). This was also the case in the male subsample for the relation with PHQ-9 levels as predictor and AEA as outcome showing that a between-person increase in PHQ-9 levels led to a significant decrease in AEA across the four timepoints ($b = -.020$, $p < .05$, 95% CI [-.035; -.005]). No other significant result was found in regard to the within-person and between-person relation between PHQ-9 scores and AEA levels in the overall sample or the female subsample (see table 2 and 3).

Either a between-person nor a within-person increase in cortisol, 2-AG/1-AG, OEA, PEA or SEA did significantly predict depression symptomatology and depression symptomatology did not predict cortisol, 2-AG/1-AG, OEA, PEA or SEA levels in the overall- and the two subsamples (all $p > .05$).

Burnout symptoms (MBI), endocannabinoides and cortisol

In the male subsample, a within person increase in MBI scores, lead to a significant increase in AEA levels ($b = .212$, $p < .05$, 95% [CI .025; .429]) over time (see table 5), however, a within person increase in AEA levels did not significantly predict MBI scores (see table 4). This relation seemed reversed when considering between-person differences for the direction of the association. However, an increase in MBI scores did not significantly predict a decrease of AEA levels ($b = -.056$, $p = .283$, 95% CI [-.158; .043]). No other significant findings were found regarding the within-person and between-person relation between MBI scores and AEA levels in the overall sample or the female subsample (see table 4 and 5).

In the overall sample a within-person increase in MBI scores lead to a significant decrease in cortisol levels ($b = -.094$, $p < .05$, 95% CI [-.184; -.006]) across time (see table 7), while a within person increase in cortisol did not predict MBI scores across time (see table 6). The aforementioned findings exhibited the same direction of effects when looking at between-person differences, but not identifying a significant effect for an increase in MBI scores predicting a decrease in cortisol levels ($b = -.001$, $p = .981$, 95% CI [-.048; .049]). No significant association was found regarding the within-person and between-person relation between MBI scores and cortisol levels in the female or the male subsample alone (see table 6 and 7).

No between-person or a within-person increases in 2-AG/1-AG, OEA, PEA or SEA did significantly predict MBI scores and MBI scores did not predict 2-AG/1-AG, OEA, PEA or SEA levels in the overall- and the male or female subsamples (all $p > .05$).

Exploratory Analyses

Cortisol did not significantly moderate the between and within person relation between AEA, 2-AG/1-AG, OEA, PEA or SEA with regard to PHQ-9 scores and vice versa. AEA, 2-AG/1-AG, OEA, PEA and SEA did not moderate the relation between the predictor cortisol and the criterion PHQ-9 and vice versa.

Cortisol significantly moderated the within person relation between MBI as predictor and 2-AG/1-AG as criterion in the overall sample ($b = .184, p < .05, 95\% \text{ CI } [.015; .346]$). Additionally, cortisol significantly moderated the relation between MBI as predictor and 2-AG/1-AG as criterion in the male subsample ($b = -8.594, p < .05, 95\% \text{ CI } [-16.141; -.583]$) but not in the female subsample ($b = .171, p = .065, 95\% \text{ CI } [-.006; .334]$). Cortisol did not significantly moderate the within person relation between 2-AG/1-AG as predictor and MBI as criterion in the overall sample ($b = .041, p = .968, 95\% \text{ CI } [-2.055; 2.006]$) or the female ($b = .036, p = .976, 95\% \text{ CI } [-2.408; 2.359]$). However, cortisol did significantly moderate the within person relation between 2-AG/1-AG as predictor and MBI as criterion in the male subsample ($b = -8.594, p < .05, 95\% \text{ CI } [-8.104; 2.302]$).

2-AG/1-AG significantly moderated the relation between the predictor MBI and the criterion cortisol in the overall sample ($b = .850, p < .01, 95\% \text{ CI } [.226; 1.487]$) and the female subsample ($b = .952, p < .01, 95\% \text{ CI } [.260; 1.614]$) but not in the male subsample ($b = .026, p = .984, 95\% \text{ CI } [-2.537; 2.440]$). This was not the case the other way around, meaning that when cortisol was the predictor and MBI was the outcome variable the relation was not moderated by 2-AG/1-AG in the overall sample ($b = 1.194, p = .865, 95\% \text{ CI } [-1.670; 3.852]$) and the female subsample ($b = 2.139, p = .158, 95\% \text{ CI } [-.772; 5.177]$). However, the aforementioned relation was significant for the male subsample ($b = -8.277, p < .05, 95\% \text{ CI } [-16.401; -.327]$).

Cortisol did not significantly moderate the within person relation between AEA, OEA, PEA and SEA with regard to MBI scores and vice versa. AEA, OEA, PEA and SEA did not moderate the relation between the predictor cortisol and the criterion MBI and vice versa.

Cortisol did not significantly moderate the between person relation between AEA, 2-AG/1-AG, OEA, PEA and SEA with regard to MBI scores and vice versa. AEA, 2-AG/1-AG, OEA, PEA and SEA did not moderate the relation between the predictor cortisol and the criterion MBI and vice versa.

Discussion

Summary

The present study is worldwide the first to examine cross-sectional and longitudinal relations on the between- and within-person level for depression and burnout symptomatology and hair cortisol and endocannabinoids. Cross-sectional comparisons reveal for depression and burnout groups reduced cortisol levels, while only for the depression group also reduced AEA levels were observed. Longitudinal multilevel analyses suggest that a within-person increase in burnout, but not depression, symptoms precedes a decrease in cortisol levels in the overall sample. In the female subsample no longitudinal associations were identified. However, in the male subsample, on the between-person level an increase in depression symptoms preceded and followed a decrease in AEA levels, while on the within-person level an increase in depression symptoms preceded and followed an increase in AEA levels. In men, also a within-person increase in burnout symptomatology preceded an increase in AEA levels. Finally, moderation analyses revealed that cortisol moderates the relation between burnout symptoms and 2-AG/1-AG over time and that 2-AG/1-AG moderates the relation between burnout symptoms and cortisol over time.

Integration of findings

The finding of reduced hair cortisol levels in the depression group as compared to the low symptom group adds to the emerging picture of generally lower hair cortisol levels in depressive disorders (Rothe et al., 2020). For example, in a study of 28 participants (4 males) the MDD group exhibited significantly lower hair cortisol levels than controls (Steudt-schmiedgen et al., 2017). Similarly, in two only female samples, reduced hair cortisol levels were identified in the MDD cases as compared to healthy controls (Behnke et al., 2021; Pochigaeva et al., 2017). However, there is conflicting literature showing increased hair cortisol levels in depressed individuals compared to controls (Dettenborn et al., 2012; Wei et al., 2015) or no differences (Gerritsen et al., 2019). A meta-analysis on hair cortisol did not identify any association between hair cortisol and mood disorders or depressive

symptomatology (Stalder et al., 2017b). Therefore, our cross-sectional data add to the mixed findings on hair cortisol and depression suggesting either additional factors to influence this association or time-dependent effects of the disease course in regard to its pathophysiology. The fact that the subjects studied were most likely at very different points in the disease course at the measurement time points may have introduced too much variance in terms of the highly adaptive glucocorticoid secretion. This is also reflected by the fact, that there emerged no significant association in longitudinal analyses between depression symptoms and cortisol levels.

In addition, a positive burnout screening at baseline was also associated with reduced cortisol levels compared to controls, and this finding was corroborated in the longitudinal analysis revealing a within-person increase in burnout symptoms to be associated with a decrease in cortisol levels across time over the entire sample. This now confirms previous findings on the within-person level, while previously a prospective association between increased work-related stress measured by the effort-reward imbalance scale and reduced hair cortisol levels at two-year follow-up on the group level was highlighted (Penz et al., 2019). Notably, two previous reports using single time point measures of hair cortisol suggested burnout to be associated with higher hair cortisol levels (Penz et al., 2018b; Wendsche et al., 2020), highlighting the importance of repeated measures analyses to map the dynamics between changes in burnout symptoms and changes in hair cortisol. It is further relevant to highlight that although within-person associations corroborated the initially identified cross-sectional negative association between burnout symptoms and hair cortisol levels on the within-person level, this was not identified in the between-person analysis. Large differences in the intercepts between persons may have obscured between-person associations with regard to the centered grand mean, whereas differences becomes more visible in the person mean centered differences (Curran and Bauer, 2011).

Based on the recognition of the functional interaction between the HPA axis and the ECS, endocannabinoids such as AEA and 2-AG/1-AG have been discussed on the one hand as potential biomarkers and on the other hand as factors that in concert with the HPA axis system may cause depression or stress-related syndromes such as burnout or as a consequence of the disorder be dysregulated (Behnke et al., 2021; Gao et al., 2020; Giacobbe et al., 2021; Hill et al., 2018). However, except for the present study, it has never been investigated whether the between- and within-person changes in depression or burnout symptoms are related to

to endocannabinoid and cortisol levels using a baseline assessment and three annual follow-up examinations. Corroborating the at baseline observed difference in AEA levels with reduced levels in the group with a positive depression screening as compared to the group with low depression symptoms, only in the male subsample this pattern was confirmed in the between-person examination. We found that in men, in general an increase in depression symptoms led to a decrease in AEA levels over time and vice versa. This findings are in line with the majority of previous reports showing lower AEA levels to be associated with depression or stress-related mental disorders (Hill et al., 2013, 2009; Hill et al., 2008b; Neumeister et al., 2013). This further suggests that in men, AEA levels may be reduced as a consequence of a depressive disorder and that a pharmacological supplementation of AEA or FAAH inhibition may achieve symptom alleviation as previously reported for social anxiety disorders or in principle for PTSD (Mayo et al., 2020; Schmidt et al., 2021). Yet, with respect to women, confounding factors such as sex hormone fluctuations might obscure this relation (Balsevich et al., 2017; Fiacco et al., 2019). Introducing more complexity with regard to the relation between the ECS and depression, recent findings suggest that a history of trauma influences basal endocannabinoid levels and CB1R availability depending on the developmental stage (Nia et al., 2019).

Focusing on hair endocannabinoids, it can be said that small sample sizes and the cross-sectional nature of previous studies limit the interpretability of existing reports suggesting a negative trend between AEA levels and depressive symptoms in 31 women (Wingenfeld et al., 2018). Similarly, in a sample of 207 individuals of the general population, a significant negative cross-sectional correlation between hair AEA levels and anxiety and burnout symptoms was identified, while also a negative trend with depression symptoms emerged (Gao et al., 2020). Furthermore, a negative correlation between PEA, OEA, SEA and PTSD symptom severity in 76 rebel war survivors was reported (Wilker et al., 2016), while Croissant et al. (2020) report for 93 male refugee minors a positive correlation between 2-AG/1-AG with depression symptoms, and Behnke et al. (2021) identify no association between hair endocannabinoids and depression status in women. Thus, the present results overall support and extend pre-clinical and clinical findings on AEA from blood serum and hair (Behnke et al., 2021; Gao et al., 2020; Hill et al., 2008; Hill et al., 2008b; Shen et al., 2019), and highlight hair AEA as potential biomarker for depression and burnout (Gao et al., 2020).

Therefore, it was unexpected to the authors that in the male subsample, within-person increases in depression symptoms significantly predicted within-person increases in AEA levels over time and vice versa, while the between-person relation was negative (see Figure 5). On the one side, this could indicate a compensatory mechanism raising AEA levels during acute periods of increased depression symptomatology, which is then followed by a general reduction of AEA secretion over time being caught by the conducted multilevel analysis (Curran and Bauer, 2011). More specifically, the positive within-person effect reflects that, on average, men tend to exhibit higher AEA levels in periods when their depression symptomatology is elevated relative to their typical stable level (Berger et al., 2018); this might be attributable to the energy-providing effect of increased AEA recruitment and CB₁R signaling when depressed men need to surmount current challenges (Balsevich et al., 2017). In contrast, the negative between-person effect reflects that, on average, men who are more depressed tend to exhibit lower AEA levels over time. This might be attributable to an exhaustion of the ECS after depressive periods of excess need of energy as similarly suggested for the HPA axis (Rothe et al., 2020; Steudte-Schmiedgen et al., 2017). These results therefore reflect a time-sensitive pattern in which the ECS system supports countermechanisms against depression establishment. However, if the increase in depression symptoms is not stopped, the ECS may eventually exhaust and release blunted AEA levels as is typical in MDD. The finding of a within-person effect in the male subsample revealing increased burnout symptoms leading to increased AEA levels over time, lends further support for the hypothesis of an identified counterregulatory mechanism of the ECS. To further test this hypothesis, however, more measurements with shorter intervals, a longer study period and an aggregation of the stress period would be needed to catch this point of AEA level reversal as a consequence of ongoing symptom burden.

The fact that in the female subsample no significant associations were identified points to additional confounding variables such as sex hormone levels or age potentially interacting with the ECS (Fiacco et al., 2018; Walther et al., 2019; Walther et al., 2019; Walther et al., 2020). However, conducting exploratory analyses controlling for birth control measures did not reveal further associations. Another route of explanation might be the low cross-sectional variance in depression and burnout symptomatology in the female subsample as well as less change over time reducing the probability to identify statistical significance. This highlights the need for larger samples to identify between- and within-person associations for

depression and burnout symptoms in relation to hair glucocorticoids and endocannabinoids in women.

Finally, our results suggest that with regard to burnout symptoms, hair 2-AG/1-AG and cortisol are significant moderators of each other, which also corroborated the previous finding that changes in 2-AG levels following stress seem to be mediated by glucocorticoids (Nia et al., 2019). However, systematic interaction effects between these two parameters should be further examined in future research.

Limitations

Limitations of the present study must be considered when interpreting the results. First, no clinical diagnostic interview or medical examination was performed at baseline. This leaves the possibility of large heterogeneity in the sample with respect to underlying diagnoses. Thus, although standardized screening instruments for depression or burnout were used, other underlying conditions causing the depression or burnout symptoms cannot be excluded. Possible somatic diseases could also have an impact on cortisol or endocannabinoid levels. Furthermore, even though the Dresden Burnout Study comprises a large overall sample, the number of subjects who participated in at least three of the four measurement time points between 2015 and 2018 is relatively small for multilevel analyses with 128 subjects. Although there is worldwide no other sample like this, longitudinal subgroup analyses are only moderately powered with sample sizes ranging from 20 to 108 subjects. This also limits the ability to test a broad set of covariates simultaneously. Finally, it is important to note the small number of male participants, although it is in this subgroup that consistent longitudinal associations between AEA level and depressive symptoms were identified.

Conclusion

Depressive disorders and burnout are complex conditions requiring in-depth analysis of time-dependent symptom and biomarker changes. The present study represents a first attempt to map cross-sectional and longitudinal between- and within-person relations between depression and burnout symptoms and hair cortisol and endocannabinoid levels. For the overall sample a within-person increase in burnout symptoms predicted a decrease in cortisol levels over time highlighting the HPA axis as a stress-physiological system that appears to enter exhaustion with reduced glucocorticoid secretion after a one-year stress period. Only

after this stressful period is terminated recovery of HPA axis activity seems possible. With regard to endocannabinoids, reduced AEA levels seem to precede and follow increased depression symptoms in men, while during acute depression phases increased AEA levels seem to be present indicative of a counterregulatory mechanisms. This further highlights AEA supplementation and FAAH inhibition as promising adjunct treatment especially in men with depression symptoms, while more research is needed in female samples examining potential confounding factors.

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Declaration of interest

All authors declare no conflict of interest.

Author contributions

Conceptualization: CK, WG, AW, MP, MW; **Recruitment and testing:** NR, MP, MW, AW; **Biochemical analyses:** WG; **Data analysis and interpretation:** AW, SW, WG; **Writing of first draft:** AW, SW, WG; **Editing of first draft:** CK, AW, WG, NR, MP, MW.

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Table 1. Sample characteristics (N=128)

	Total sample	Women	Men	<i>t</i> (χ^2)	<i>p</i>
	n = 128	n = 108	n = 20		
Age, M (SD)	44.96 (12.04)	44.40 (12.13)	48 (11.35)		
BMI, M (SD)	25.79 (4.81)	25.47 (4.71)	27.55 (5.07)		
Tobacco smokers, n (%)	20 (15.63)	18 (16.67)	2 (10)	0.584	.747
Weekly alcohol consumers, n (%)	25 (19.531)	18 (16.67)	7 (35)	5.493	.905
Medication intake, n (%)	42 (32.81)	38 (35.19)	4 (20)	1.765	.184
Antidepressants intake, n (%)	11 (8.59)	9 (8.33)	2 (10)	.060	.807
Thyroid supplementation, n (%)	26 (20.31)	25 (23.15)	1 (5)	3.434	.064
Birth control, n (%)	17 (13.28)	17 (15.74)	0 (0)		
Multiple medication, n (%)	19 (14.84)	16 (14.81)	3 (15)	0.000	.983
Chronic disease, n (%)	42 (32.81)	38 (35.19)	4 (20)	1.765	.184
Psychological disorder, n (%)	22 (17.19)	19 (17.59)	3 (15)	.080	.778
Depressive Symptoms (PHQ-9), M (SD)	8.35 (5.31)	8.51 (4.89)	7.74 (6.83)	-.567	.572
Positive screening for current major depressive syndrome (MDD) PHQ-9 $\geq$ 10, n (%)	30 (23.44)	25 (23.15)	5 (25)	.389	.534
Burnout Symptoms (MBI-GS), M (SD)	1.90 (1.16)	1.87 (1.17)	2.02 (1.09)	.530	.597
Positive screening for burnout MBI-GS $\geq$ 3.5, n (%)	19 (14.84)	18 (16.67)	1 (5)	1.817	.178
Cortisol (pg/mg), M (SD)	13.54 (46.63)	14.97 (51.15)	6.67 (6.92)	-.509	.613
Cortisol (log), M (SD)	.72 (.38)	.72 (.38)	.67 (.36)	-.391	.700
Anandamide (AEA; pg/mg), M (SD)	.75 (.75)	.69 (0.70)	.98 (.97)	.964	.341
Anandamide (AEA; log), M (SD)	-.32 (.41)	-.34 (.40)	-.25 (.51)	.557	.580
Arachidonoyl glycerol (AG-1 & AG-2; pg/mg), M (SD)	33.21 (13.49)	32.88 (13.97)	34.85 (11.31)	.417	.678
Arachidonoyl glycerol (AG-1 & AG-2; log), M (SD)	1.48 (.19)	1.48 (.20)	1.52 (.13)	.725	.471
Oleylethanolamide (OEA; pg/mg), M (SD)	1629.28 (1447.88)	1611.72 (1412.86)	1717.07 (1692.48)	.208	.836
Oleylethanolamide (OEA; log), M (SD)	3.05 (.39)	3.05 (.38)	3.03 (.47)	-.143	.887

Longitudinal cortisol, AEA, depression and burnout relations

Palmitoylethanolamide (PEA; pg/mg), M (SD)	1927.83 (1424.82)	1988.80 (1499.26)	1622.98 (972.95)	-.738	.463
Palmitoylethanolamide (PEA; log), M (SD)	3.17 (.31)	3.18 (.32)	3.12 (.31)	-.565	.575
Stearoylethanolamine (SEA; pg/mg), M (SD)	381.55 (303.02)	386.66 (320.42)	355.96 (205.15)	-.290	.773
Stearoylethanolamine (SEA; log), M (SD)	2.47 (.31)	2.47 (.32)	2.48 (.28)	.078	.938

Note: N: sample size, M: mean, SD: standard deviation, BMI: body mass index; PHQ-9: Patient Health Questionnaire 9, MBI-GS: Maslach Burnout Inventory-General Survey.

Table 2. Results of random intercept models predicting symptoms of depression (PHQ-9)

	PHQ-9 (general sample)				PHQ-9 (female subsample)				PHQ-9 (male subsample)			
Fixed effects	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>
Intercept	1.709	3.424	.499	[-5.270; 8.743]	5.806 [†]	3.136	1.851	[-.610; 12.560]	-7.728	9.354	-.826	[-28.183; 10.652]
AEA pmc	1.901	2.193	.867	[-2.234; 6.149]	-.046	2.190	-.021	[-4.283; 4.235]	27.504*	8.630	3.187	[9.724; 44.038]
AEA gmc	-.460	2.029	-.227	[-4.263; 3.329]	1.082	1.965	.551	[-2.839; 4.791]	-24.389*	8.488	-2.873	[-41.368; -6.409]
Age	-.018	.049	-.365	[-.114; .082]	.019	.047	.407	[-.088; .112]	-.092	.204	-.453	[-.473; .315]
Sex	.878	1.332	.660	[-1.697; 3.424]								
BMI	.197	.122	1.613	[-.047; .440]	.011	.129	.084	[-.250; .270]	.537 [†]	.275	1.950	[-.009; 1.097]
Alcohol consumption	.742	.517	1.434	[-.253; 1.790]	.678	.524	1.293	[-.372; 1.734]	2.433	1.564	1.555	[-.484; 5.509]
Random effects	<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>		
Intercept	17.66	4.202		[3.495; 5.078]	14.054	3.749		[3.007; 4.536]	14.044	3.748		[1.972; 6.136]
Residual	6.28	2.506		[2.179; 2.758]	6.788	2.605		[2.244; 2.932]	4.264	2.065		[1.512; 2.579]
Modell fit												

ICC	.76	.72	.87
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Note: Multilevel regression models were conducted controlling for age, sex, BMI, medication intake, and alcohol consumption, gmc = grand mean centered, pmc = person mean centered, B = unstandardized regression coefficient, SE = standard error, SD = standard deviation, v = variance, ICC = intraclass correlation, † indicates associations below $p = .1$, * indicates associations below $p = .05$, ** indicates associations below $p = .01$.

Table 3. Results of random intercept models predicting AEA levels

Fixed effects	AEA (general sample)				AEA (female subsample)				AEA (male subsample)			
	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>
Intercept	-.403*	.200	-2.021	[-.807; -.008]	-.278	.203	-1.365	[-.690; .112]	-.827**	.297	-2.782	[-1.444; -.212]
PHQ-9 pmc	.012	.010	1.150	[-.008; .031]	.001	.012	.079	[-.021; .023]	.061**	.021	2.909	[-.021; .103]
PHQ-9 gmc	-.001	.007	-.089	[-.014; .013]	.006	.008	.753	[-.011; .022]	-.020*	.008	-2.543	[-.034; -.004]
Age	.003	.003	1.133	[-.003; .008]	.002	.003	.825	[-.003; .008]	.004	.007	.531	[-.010; .017]
Sex	-.004	.074	-.055	[-0.142; .146]								
BMI	.001	.007	.200	[-.013; .015]	-.001	.008	-.071	[-.017; .015]	.006	.011	.548	[-.015; .028]
Alcohol consumption	-.050 ^t	.029	-1.729	[-.107; .006]	-.078*	.032	-2.392	[-.139; -.016]	.086	.049	1.750	[-.008; .180]
Random effects	<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>		
Intercept	.038	.196		[.141; .254]	.042	.205		[.146; .268]	.000	.000		[.000; .166]
Residual	.064	.253		[.222; .279]	.065	.255		[.219; .285]	.052	.227		[.165; .272]
Modell fit												
ICC	.39				.42				.24			

Note: Multilevel regression models were conducted controlling for age, sex, BMI, medication intake, and alcohol consumption, gmc = grand mean centered, pmc = person mean centered, B = unstandardized regression coefficient, SE = standard error, SD = standard deviation, v = variance, ICC = intraclass correlation, ^t indicates associations below $p = .1$, * indicates associations below $p = .05$, ** indicates associations below $p = .01$.

Table 4. Results of random intercept models predicting burnout symptoms (MBI-GS)

	MBI-GS (general sample)				MBI-GS (female subsample)				MBI-GS (male subsample)			
Fixed effects	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>
Intercept	-.047	.813	-.058	[-1.622; 1.606]	.599	.823	.729	[-1.072; 2.222]	-.373	2.231	-.167	[-4.730; 3.808]
AEA pmc	-.137	.516	-.266	[-1.174; .837]	-.523	.558	-.938	[-1.575; .535]	3.096	2.053	1.508	[-.953; 7.302]
AEA gmc	.271	.481	.564	[-.669; 1.230]	.538	.515	1.045	[-.493; 1.546]	-2.472	2.025	-1.221	[-6.664; 1.357]
Age	.003	.012	.272	[-.020; .027]	.008	.012	.669	[-.016; .033]	-.024	.049	-.495	[-.118; .069]
Sex	.401	.317	1.267	[-.200; 1.014]								
BMI	.071*	.029	2.459	[.015; .127]	.051	.034	1.525	[-.018; .121]	.122†	.066	1.855	[-.014; .250]
Alcohol consumption	.051	.123	.419	[-.187; .279]	.077	.138	.557	[-.211; -.346]	.159	.373	.426	[-.576; .857]
Random effects	<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>		
Intercept	1.015	1.007		[.840; 1.195]	1.027	1.013		[.823; 1.217]	.812	.901		[.503; 1.421]
Residual	.307	.554		[.488; .615]	.330	.575		[.496; .640]	.203	.451		[.326; .562]

Modell fit

ICC	.66	.62	.85
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Note: Multilevel regression models were conducted controlling for age, sex, BMI, medication intake, and alcohol consumption, gmc = grand mean centered, pmc = person mean centered, B = unstandardized regression coefficient, SE = standard error, SD = standard deviation, v = variance, ICC = intraclass correlation, ^t indicates associations below $p = .1$, * indicates associations below $p = .05$, ** indicates associations below $p = .01$.

Table 5. Results of random intercept models predicting AEA levels

	AEA (general sample)				AEA (female subsample)				AEA (male subsample)			
Fixed effects	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>
Intercept	-.364 ^t	.201	-1.809	[-.824; -.030]	-.246	.208	-1.185	[-.658; .209]	-.673 ^t	.342	-1.967	[-1.370; -.021]
MBI-GS pmc	.019	.047	.407	[-.071; .116]	-.025	.052	-.486	[-.132; .086]	.212 [*]	.104	2.040	[-.025; .429]
MBI-GS gmc	.008	.029	.284	[-.053; .063]	.029	.033	.877	[-.041; .095]	-.056	.049	-1.147	[-.157; .042]
Age	.003	.003	1.156	[-.003; .008]	.002	.003	.815	[-.004; .008]	.008	.007	1.117	[-.006; .023]
Sex	-.010	.074	-.129	[-.160; .144]								
BMI	-.000	.007	-.017	[-.013; .015]	-.002	.008	-.250	[-.019; .013]	-.006	.012	-.516	[-.029; .019]
Alcohol consumption	-.050 ^t	.029	-1.744	[-.107; .007]	-.075 [*]	.032	-2.344	[-.137; -.010]	.058	.055	1.051	[-.055; .164]
Random effects	<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>		
Intercept	.038	.194		[.142; .250]	.041	.204		[.147; .268]	.003	.056		[.000; .201]
Residual	.065	.254		[.225; .282]	.065	.256		[.218; .285]	.058	.240		[.177; .291]
Modell fit												
ICC	.39				.42				.14			

Note: Multilevel regression models were conducted controlling for age, sex, BMI, medication intake, and alcohol consumption, gmc = grand mean centered, pmc = person mean centered, B = unstandardized regression coefficient, SE = standard error, SD = standard deviation, v = variance, ICC = intraclass correlation, [†] indicates associations below $p = .1$, * indicates associations below $p = .05$, ** indicates associations below $p = .01$.

Table 6. Results of random intercept models predicting burnout symptoms

	MBI-GS (general sample)				MBI-GS (female subsample)				MBI-GS (male subsample)			
Fixed effects	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>
Intercept	-.058	.811	-.072	[-1.594; 1.545]	.667	.837	.797	[-1.070; 2.391]	-1.666	1.981	-.841	[-5.732; 2.484]
Cortisol pmc	-.361	.529	-.683	[-1.421; .619]	-.145	.597	-.242	[-1.310; .991]	-.786	1.323	-.594	[-3.342; 1.817]
Cortisol gmc	-.016	.507	-.032	[-.973; 1.017]	-.213	.571	-.374	[-1.287; .870]	.315	1.292	.244	[-2.224; 2.811]
Age	.006	.011	.580	[-.014; .026]	.005	.012	.419	[-.019; .029]	.017	.029	.601	[-.018; .073]
Sex	.495 [†]	.297	1.666	[-.098; 1.067]								
BMI	.063 [*]	.029	2.183	[.008; .120]	.053	.033	1.588	[-.010; .120]	.129	.082	1.570	[-.038; .283]
Alcohol consumption	.031	.114	.272	[-.209; .265]	.071	.126	.563	[-.164; .320]	-.310	.347	-.893	[-1.055; .394]
Random effects	<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>		
Intercept	.980	.990		[.810; 1.169]	.984	.992		[.809; 1.210]	1.129	1.063		[.634; 1.670]
Residual	.313	.560		[.494; .612]	.345	.587		[.514; .656]	.191	.437		[.326; .528]
Modell fit												

ICC	.66	.62	.85
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Note: Multilevel regression models were conducted controlling for age, sex, BMI, medication intake, and alcohol consumption, gmc = grand mean centered, pmc = person mean centered, B = unstandardized regression coefficient, SE = standard error, SD = standard deviation, v = variance, ICC = intraclass correlation, [†] indicates associations below $p = .1$, * indicates associations below $p = .05$, ** indicates associations below $p = .01$.

Table 7. Results of random intercept models predicting cortisol levels

Fixed effects	Cortisol (general sample)				Cortisol (female subsample)				Cortisol (male subsample)			
	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>95% CI</i>
Intercept	.419*	.188	2.226	[.041; .809]	.405*	.188	2.156	[.046; .773]	.231	.495	.467	[-.687; 1.196]
MBI-GS pmc	-.094*	.044	-2.115	[-.184; -.006]	-.072	.048	-1.486	[-.163; .018]	-.199	.119	-1.669	[-.433; .054]
MBI-GS gmc	-.001	.027	-.023	[-.051; .047]	-.012	.029	-.430	[-.067; .037]	.027	.070	.383	[-.114; .160]
Age	-.004	.002	-1.593	[-.008; .001]	-.003	.003	-1.150	[-.008; .002]	-.006	.006	-.981	[-.019; .006]
Sex	-.049	.066	-.752	[-.181; .084]								
BMI	.143*	.006	-2.211	[.002; .027]	.011	.007	1.486	[-.004; .025]	.032	.018	1.740	[-.002; .068]
Alcohol consumption	-.047 ^t	.025	1.893	[.000; .101]	.055*	.027	2.046	[-.000; -.104]	-.030	.081	-.367	[-.187; .133]
Random effects	<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>			<i>v</i>	<i>SD</i>		
Intercept	.026	.161		[.105; .212]	.024	.154		[.083; .216]	.040	.199		[.040; .346]
Residual	.078	.280		[.249; .308]	.081	.285		[.252; .316]	.066	.260		[.197; .310]
Modell fit												

ICC	.34	.29	.49
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Note: Multilevel regression models were conducted controlling for age, sex, BMI, medication intake, and alcohol consumption, gmc = grand mean centered, pmc = person mean centered, B = unstandardized regression coefficient, SE = standard error, SD = standard deviation, v = variance, ICC = intraclass correlation, ^t indicates associations below $p = .1$, * indicates associations below $p = .05$, ** indicates associations below $p = .01$.

Figure 1. Comparison between the depressive syndrome group and controls regarding cortisol and endocannabinoids at baseline

Figure 2. Comparison between the burnout syndrome group and controls regarding cortisol and endocannabinoids at baseline

Figure 3. Depiction of longitudinal change patterns for the depression syndrome group and controls regarding cortisol and endocannabinoids

Note: Error bars indicate CI95%. T1-T4 represent measurement time points 1 till 4 with annual intervals.

Figure 4. Depiction of longitudinal change patterns for the burnout syndrome group and controls regarding cortisol and endocannabinoids

Note: Error bars indicate CI95%. T1-T4 represent measurement time points 1 till 4 with annual intervals.

Figure 5. Between-person (A) and within-person (B) relation between depression symptoms and AEA (log) for the male subsample