



Long-term glucocorticoid levels measured in hair in patients with depressive and anxiety disorders

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ABSTRACT

Background: Depressive and anxiety disorders have been linked to a dysregulated hypothalamus-pituitary-adrenal (HPA)-axis. Hair cortisol levels (HairF) reflect integrated long-term cortisol regulation and are therefore promising endocrine markers of chronic (psychological and physical) stress.

Our aim was to assess hair cortisol levels in persons with a depressive and/or anxiety disorder and to compare their levels with that of persons in remission and healthy controls.

Methods: Data from 1166 participants of the Netherlands Study of Depression and Anxiety (NESDA) were used, including 266 participants with a recent (1-month) diagnosis of a depressive and/or anxiety disorder, 655 participants with a diagnosis in remission, and 245 healthy controls. HairF was measured in the proximal three cm of scalp hair, using LC-MS/MS.

Results: Compared to the healthy controls no differences on HairF or HairE levels were found for depressive and anxiety disorders alone. However the presence of a comorbid depressive and anxiety disorder was significantly associated with increased HairF levels ($\beta = 0.07$; $p = .031$), as was the severity of depressive symptoms ($\beta = 0.06$; $p = .029$), but no differences were found on HairE nor the HairF:HairE ratio.

Conclusions: Persons with current diagnosis of comorbid depression and anxiety show moderately higher levels of cortisol than patients with only depression or anxiety, or patients in remission and healthy controls, which may be indicative of a chronic state of hyperactivation of the HPA axis.

1. Introduction

Dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis may result in increased or decreased levels of its end-product, cortisol. A disproportionate change in cortisol levels is one of the main hypothesized pathophysiological mechanisms underlying depressive and anxiety disorders (Casper et al., 1988; Elnazer and Baldwin, 2014; Hilbert et al., 2014; Vreeburg et al., 2009, 2010). However, findings have been inconsistent regarding relative hyper- or hypocortisolism in different (subtypes of) disorders. So far, most studies examining cortisol in depressive disorders observed higher levels of cortisol (see meta-analyses: (Belvederi Murri et al., 2014; Knorr et al., 2010)), whereas both higher and lower levels have been found in relation to anxiety

disorders. For instance, higher levels of cortisol have been found for panic disorder and agoraphobia (Vreeburg et al., 2010) but lower levels have also been found for social phobia and PTSD (Zorn et al., 2017). Moreover, due to the high comorbidity between depression and anxiety, many studies include samples of comorbid patients and it may well be that this comorbid group has a distinct cortisol pattern from depression and anxiety alone (Morris et al., 2012).

Most of the preceding studies measured cortisol in urine, blood or saliva. One shortcoming of these methods is that they only reflect cortisol levels over a relatively short time period, i.e. minutes to several days, thereby not reflecting chronic exposure to cortisol excretion, which likely is more relevant in terms of health effects. Another question that remains to be answered is whether altered cortisol levels

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represent a trait or state effect in depressive (Lok et al., 2012) and anxiety disorders. One possibility to shed more light on these issues is the use of long-term cortisol concentrations as measured in hair segments.

Hair cortisol concentrations (HairF) are assumed to reflect integrated long-term (e.g. 1 cm of hair is assumed to represent 1 month) cortisol levels, and have been proposed as a promising endocrine marker of chronic stress. Indeed, higher HairF in patients with depression compared to healthy controls have been reported (Caparros-Gonzalez et al., 2017; Dettenborn et al., 2012a,b; Pochigaeva et al., 2017; Wei et al., 2015), while, other studies showed no difference (Herane-Vives et al., 2018; Hinkelmann et al., 2013; Kuehl et al., 2015). Research on HairF and anxiety disorders has also reported differences between cases and controls; e.g. posttraumatic stress disorder (PTSD) has been associated with increased HairF in the months after the trauma, followed by decreased HairF in the longer run (for review see (Steudte-Schmiedgen et al., 2016)). And a small recent study on hair cortisol levels among patients with MDD and/or GAD found that MDD patients show lower cortisol levels than controls, GAD and comorbid patients (Steudte-Schmiedgen et al., 2016). To summarize, first studies on hair cortisol levels have been published, but there still is a considerable lack of consistent information on the relationship with depressive and anxiety disorder characteristics such as age of onset, duration of disorder, severity, and use of antidepressants. Furthermore, most studies involved small samples (many less than $n = 50$), which may partially explain discrepant results across studies.

The development of new techniques to measure HairF has led to the possibility to measure other steroid hormones in scalp hair as well. One of these other hormones is cortisone, the inactive form of cortisol. The enzyme 11β -hydroxysteroid dehydrogenase type 1 (11β HSD-1) converts cortisone into active cortisol, whereas its counterpart 11β -hydroxysteroid dehydrogenase type 2 (11β HSD-2) converts active cortisol to inactive cortisone. The assessment of hair cortisone (HairE) in parallel to HairF has been postulated to provide even more insight into the cumulative amount of active and inactive glucocorticoids in the body, because for instance higher levels of free cortisol, could be due to a decreased conversion to cortisone rather than an increase in cortisol output. As such HairE levels may provide a useful and robust marker of long-term HPA axis activity (Stalder et al., 2013). Apart from the absolute levels of HairF and HairE, the cortisol-to-cortisone ratio is also of interest, as it may reflect the 11β HSD-activity (Zhang et al., 2013). The importance of this ratio in stress-related research has been emphasized by studies using urinary measures showing an altered ratio under stress (Plenis et al 2011) and demonstrating an enhanced ratio in depressed patients (Dekker et al 2012; Romer et al 2009), suggestive of an increased conversion to active cortisol by 11β HSD-1.

Measurement of hair cortisone concentrations (HairE) have been shown by our group and by others to be easily measurable and to correlate with HairF (Raul et al., 2004; Stalder et al., 2013; Staufenbiel et al., 2015; Zhang et al., 2013). Until now, no study has investigated HairF, HairE, the ratio HairF/HairE in relation to (characteristics of) depressive and anxiety disorders (and their comorbidity) while accounting for diverse covariates and using a large sample. Insight into the amounts of long-term cortisol and cortisone, as well as their ratio, may give new insights into the underlying pathophysiology of the HPA axis and its role in depressive and anxiety disorders.

In the present study, we used data from the Netherlands Study of Depression and Anxiety (NESDA) to examine whether there are differences in HairF, HairE and their ratio (HairF/HairE) between healthy control subjects and subjects with a depressive disorder or an anxiety disorder. By comparing healthy subjects, patients in remission and patients with current disorders we will be able to provide insight into state versus trait associations and we will also examine the impact of specific characteristics, such as symptom severity, age of onset and medication use, on the association between psychopathology and hair glucocorticoid levels.

2. Materials and methods

2.1. Study sample

Data were used from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal cohort study on the predictors, course and consequences of depressive and anxiety disorders. The NESDA sample consisted of 2981 participants aged 18–65 years at inclusion, comprising persons with no depressive or anxiety disorder, persons who have had a disorder in the past, and persons with a current depressive and/or anxiety disorder. A detailed description of the NESDA study rationale, design, and sampling procedure can be found elsewhere (Penninx et al., 2008). The research protocol was approved by the Ethical Committee of the participating centres, and all participants provided written informed consent. Hair data collection was added to NESDA's 6-year follow-up assessment, in which 2256 participants of the initial 2981 sample participated (75.68%). Therefore, the 6-year follow-up wave is considered baseline for the present analysis, and all determinants considered were measured at that wave. For the present study, from the 2256 participants, we selected all participants that had sufficient hair on the posterior vertex position of the head, the willingness to participate, and a hair sample weight of at least 5 mg ($n = 1677$). The participants filled in a self-developed questionnaire on their hair (treatment) characteristics. Psychiatric diagnoses were defined on the basis of the Composite International Diagnostic Interview (CIDI, versions 2.1) (Wittchen et al., 1991), which classifies diagnoses according to the DSM-IV criteria. Exclusion criteria were the diagnosis of bipolar disorder within the past year ($n = 22$), use of lithium ($n = 11$), and current use of systemic or local corticosteroids in the past three months ($n = 320$), resulting in a sample of 1264 subjects. Our final study sample consisted of 1166 subjects, due to measurement errors in hair cortisol (see paragraph below).

2.2. HairF and HairE measurement

During the visit at the research facility, hair strands of approximately 100 hairs were cut as close as possible from the scalp from a posterior vertex position. The most proximal three cm of hair were used for analysis. Based on a mean hair growth rate of one cm per month (Wenning, 2000), hair samples reflect the cumulative cortisol and cortisone secretion of the previous three months. Hair samples were weighed, finely cut with surgical scissors, and washed with 1.0 mL of LC-MS grade isopropanol for two minutes. The extraction and subsequent analysis of cortisol and cortisone by liquid chromatography-tandem mass spectrometry has extensively been described elsewhere (Noppe et al., 2015). The inter-day variation in the present study was $\leq 8.3\%$ for cortisol and $\leq 4.8\%$ for cortisone (Staufenbiel et al., 2015). Steroid peak integrations were reviewed and manually integrated by two independent persons when automated peak integration feature incorrectly or partially integrated peaks. In 98 individuals, HairF or HairE levels could not be determined due to contamination by other hormones (e.g. other steroids and metabolites) in the hair sample. The analyses were successfully performed in 1166 participants, rendering our final study sample.

2.3. Psychiatric characteristics

The DSM-IV Composite International Diagnostic Interview (CIDI) was used to assess diagnoses of depressive disorders (dysthymia, MDD) and anxiety disorders (social phobia, panic disorder, agoraphobia, panic disorder with agoraphobia, and GAD).

To investigate whether HairF and HairE levels were different between participants with a current diagnosis, participants in remission, and healthy controls, we first created three groups of subjects. The first group consisted of participants with a current (at least 1 month) depressive and/or anxiety disorder diagnosis ($n = 266$). The second group

consisted of participants with no current (at least 1 month), but in remission of depressive and/or anxiety disorder ($n = 655$). The third group comprised psychologically healthy subjects with no lifetime history of either depressive or anxiety disorders and served as control group ($n = 245$). Within the first and second group, participants could be further divided into three subgroups; 1) participants with only a depressive disorder, 2) participants with only an anxiety disorder, or 3) participants with both a depressive and anxiety disorder. This third subgroup is referred to as “participants with comorbid MDD/Anx (Major depressive disorder/ Anxiety) disorders”, meaning that both depressive and anxiety disorders are simultaneously diagnosed within a participant. Participants in this group are excluded from the groups of participants with only a depressive or anxiety disorder.

The total score of the 30-item self-report version of the Inventory for Depressive Symptomatology (IDS) was used to measure the severity of depressive symptoms (range: 0–84). The total score of the Beck Anxiety Inventory (BAI) was used to assess the severity of anxiety symptoms (range: 0–63). Additionally, the Fear Questionnaire (FQ) was used as an indicator of phobic symptoms (range: 0–120) and the total score of the childhood trauma questionnaire (CTQ) was used as a measure of childhood traumatization (range: 0–100).

To account for the use of antidepressants, we distinguished tricyclic antidepressants (TCA, ATC-code N06AA), selective serotonin reuptake inhibitors (SSRI, ATC-code N06AB), and other antidepressants (ATC-code N06AF/N06AX). Whether respondents used antidepressants was based on drug container inspection of all drugs used in the past month at baseline and classified according to the World Health Organization Anatomical Therapeutic Chemical (ATC) classification. Duration of symptoms within the previous two years was assessed with a life chart.

2.4. Covariates

We previously described the effects of age, diagnosis of diabetes mellitus (both type 1 and type 2), hair washing frequency (0–3 times/week, > 3 times/week), and season (winter, spring, summer, fall) reflected in hair sample on HairF and HairE levels (Staufenbiel et al., 2015). These will therefore be used as covariates in this study. Additionally, we included sex and hair treatment (for details see Table 1) as covariates, as these factors were associated with HairF and/or HairE in other studies (Dettenborn et al., 2012a,b; Staufenbiel et al., 2014). Other covariates such as waist circumference, smoking, and alcohol intake were not found to be related to HairF and HairE levels in our previous study (Staufenbiel et al., 2015) therefore not considered in our analyses.

2.5. Statistical analyses

For descriptive analyses, HairF and HairE are provided in pg/mg and are reported as median (Mdn) and interquartile range (IQR). For inferential analyses, HairF and HairE values were subjected to a logarithmic transformation to achieve normal distribution. Other data, unless otherwise indicated, is presented in original units with mean (M) and standard deviation (SD), or in sample size (n) and percentage (%). Baseline characteristics of the three groups were compared using χ^2 and analysis of variance statistics. Differences between HairF and HairE levels of groups of participants were conducted with univariate analyses of covariance (ANCOVA) and with linear regression analyses. Results are derived from partially-adjusted (adjusted for sex and age) and as fully-adjusted (adjusted for all covariates) analyses. The significance level was set at $p < 0.05$. All analyses were conducted using SPSS 20.0 for Windows (IBM, Chicago, Illinois). For significant findings, effect sizes were calculated with Cohen's d between healthy controls and the group in question.

Of the 1166 participants, $n = 1075$ provided a full dataset without any missing values on covariates. To overcome possible selection bias due to missing data we applied multiple imputation (Donders et al.,

2006). Assuming a missing at random approach five imputed datasets were created. All characteristics were used as predictors to impute missing values, and the observed minimum and maximum values of the original full dataset were used as constraints. When running analyses on imputed datasets, analyses are performed for each separate imputed dataset and over each dataset the results are pooled. These pooled estimates of the five imputed datasets are reported.

3. Results

3.1. Sample characteristics

Characteristics across groups are presented in Table 1. A total of 245 subjects had no current nor lifetime diagnoses (healthy controls), 655 participants were in remission (including 165 participants with remitted depressive disorder; 100 participants with remitted anxiety disorder and 390 participants with remitted comorbid depressive and anxiety disorders), and 266 participants presented with a current (past one month) diagnosis of depressive disorder ($n = 78$), anxiety disorder ($n = 122$), or comorbid both depressive and anxiety disorder ($n = 66$).

As expected, participants with a current diagnosis had higher scores on the psychiatric questionnaires and higher use of antidepressants than had participants in remission, who in turn scored higher on psychiatric questionnaires and use of antidepressants than healthy controls.

3.2. Median hair cortisol levels

The median HairF of the sample was 3.26 (2.20–5.47) pg/mg hair, whereas the median HairE of this sample was 10.68 (7.58–15.36) pg/mg hair. HairF and HairE showed a significant positive linear correlation with each other, $r = .58$, $p < .001$.

3.3. Long-term glucocorticoids in relation to remission and current diagnoses

Comparing HairF, HairE and the ratio HairF/HairE between healthy controls, participants in remission, participants with current diagnoses we found no significant difference on any of these parameters ($F_{(2, 1153)} = 1.217$, $p = .296$ for HairF, $F_{(2, 1153)} = 1.366$, $p = .255$ for HairE, and $F_{(2, 1153)} = .355$, $p = .715$ for HairF/HairE, respectively).

As participants in remission did not show altered hair levels, we investigated whether the types of remitted disorders (remitted depressive disorder, remitted anxiety disorder and remitted comorbid depressive and anxiety disorder) were differently associated with any outcome parameters. Analyses showed no association between subgroups and altered HairF, HairE, or HairF/HairE ($p > .10$).

Regarding current diagnoses, a current depressive disorder ($n = 78$) and a current anxiety disorder ($n = 122$) were not associated with altered HairF, HairE, or HairF/HairE, when compared to healthy controls (all $p > .10$). A current comorbid depressive and anxiety diagnosis ($n = 66$) was however associated with elevated HairF ($\beta = .067$, $p = .031$, $d = .33$) but not with HairF/HairE ($\beta = 0.055$, $p = .086$, $d = .34$) nor HairE ($p > .10$) compared to healthy controls, other current diagnoses and participants in remission. Fig. 1 shows the relation between HairF levels and subgroups.

The results from the partially and fully adjusted linear regression models for HairF, HairE, and HairF/HairE in relation to disorder status are shown in Table 2.

3.4. Long-term glucocorticoids in relation to severity measures

Table 2 shows all associations with the severity measures of depressive and anxiety disorders and HairF, HairE and their ratio, we will highlight some. The severity of depressive symptoms (indicated by the IDS total score) was associated with HairF in the whole sample of

Table 1
Descriptive information of the study population.

Characteristics	Healthy controls (n = 245)	Patients Current MDD (N = 78)	Current Anxiety (N = 122)	Current Comorbid MDD/Anxiety (N = 66)	Remitted MDD/Anxiety (N = 655)	p-value
Sociodemographics						
Female sex N (%)	155 (63%)	46 (59%)	106 (87%)	38 (58%)	519 (79%)	< 0.001
Mean age (SD)	46 (15)	50 (13)	47 (12)	48 (12)	47 (13)	0.17
Health and lifestyle indicators						
Diabetes mellitus N (%)	5 (2%)	2 (3%)	6 (4%)	2 (4%)	28 (4%)	0.48
Menopause N (%)	64 (42%)	22 (41%)	41 (40%)	18 (41%)	208 (40%)	0.97
Hair(treatment) characteristics N (%)						
Hair treatment in past 3 months						0.03
Dyed	12 (5%)	3 (5%)	11 (9%)	3 (6%)	61 (9%)	
Bleached	65 (27%)	18 (23%)	51 (42%)	18 (27%)	207 (32%)	
Permed	2 (1%)	0 (0%)	2 (1%)	4 (6%)	8 (2%)	
Hair washing frequency (> 3/week)	178 (73%)	38 (49%)	98 (80%)	36 (55%)	470 (72%)	0.78
Season represented in hair						0.07
Winter	43 (18%)	24 (31%)	35 (29%)	16 (24%)	178 (27%)	
Spring	60 (24%)	17 (22%)	27 (22%)	21 (32%)	154 (23%)	
Summer	67 (27%)	12 (15%)	34 (28%)	15 (23%)	166 (25%)	
Autumn	78 (32%)	25 (32%)	26 (21%)	14 (21%)	184 (28%)	
Psychiatric Aspects						
Mean IDS (SD)	5 (5)	29(11)	20 (10)	34 (11)	15 (12)	< 0.001
Mean BAI (SD)	3(4)	14(10)	14 (9)	20 (11)	10 (9)	< 0.001
Mean FQ (SD)	7.6 (8.4)	25 (18)	28 (18)	40 (19)	18 (17)	< 0.001
Mean CTQ (SD)	33 (10)	47(17)	43 (14)	45 (14)	40 (13)	< 0.001
Mean age of onset (SD)	NA	41 (17)	35 (22)	31 (16)	35(15)	0.35
Mean duration of symptoms in months (SD)	NA	14(18)	19 (17)	17 (18)	7 (10)	< 0.001
Use of antidepressants N(%)						< 0.001
TCA	0	4 (5%)	4 (3%)	8 (12%)	15 (2%)	
SSRI	1 (1%)	11 (14%)	27 (22%)	10 (15%)	81 (12%)	
Other antidepressants	0	15 (19%)	10 (8%)	10 (15%)	25 (4%)	

The reported p-value is for comparison between all groups; ANOVA for continuous and Chi-squared for categorical variables. IDS, Inventory of Depressive Symptomatology; BAI, Beck Anxiety Inventory; FQ, Fear Questionnaire; CTQ, Childhood Trauma Questionnaire; TCA, Tricyclic Antidepressant; SSRI, Selective Serotonin Reuptake Inhibitor; AD, antidepressants.

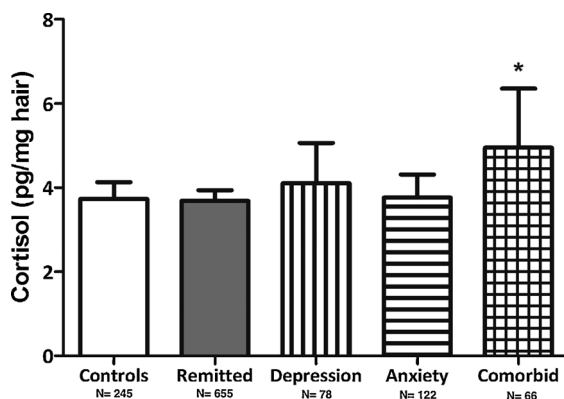


Fig. 1. HairF values for healthy controls, participants in remission, a current depressive disorder, a current anxiety disorder, and a current comorbid Major Depressive Disorder (MDD)/ Anxiety(Anx) disorder (participants with both a depressive and an anxiety disorder). Participants in the comorbid MDD/Anx group were not included in the groups of participants with only a depressive or anxiety disorder. HairF levels are expressed as geometric mean with 95% CI. *, $p < .05$ in the fully-adjusted analyses, comparison made to all other groups.

participants ($\beta = .063$, $p = .029$), but the severity of anxiety (indicated by the BAI total score) was only trend significantly associated with HairF ($\beta = 0.052$, $p = .069$) (Fig. 2). HairE and the ratio HairF/HairE was also not associated with the severity of depression (IDS), anxiety (BAI), and phobic symptoms (FQ) ($\beta = 0.052$ – 0.057 , all $p > 0.05$).

3.5. Explorative analyses on subtypes of disorders

In explorative subgroup analyses (not in the Table), we studied

current subtypes of depressive disorders (major depressive disorder, $n = 43$; dysthymia, $n = 17$; both major depressive disorder and dysthymia, $n = 18$) and of anxiety disorders (social phobia, $n = 29$; panic disorder with agoraphobia, $n = 19$; panic disorder without agoraphobia, $n = 10$; agoraphobia, $n = 28$; generalized anxiety disorder, $n = 15$; and more than one anxiety disorder, $n = 21$). The presence of current social phobia and the presence of more than one current anxiety disorder simultaneously were associated with increased HairE ($\beta = .074$, $p = .01$, $d = .53$, and $\beta = .058$, $p = .041$, $d = .24$, respectively). No other significant associations emerged (all $p > .10$).

3.6. Long-term glucocorticoids in relation to disorder characteristics

In participants with a current diagnosis of a depressive and/or anxiety disorder ($n = 266$), age of onset and duration of psychopathology were not associated with HairF, HairE, and the ratio HairF/HairE (all $p > .10$) (not in the Table).

Examining the relation of antidepressant medication revealed that the use of SSRIs was associated with increased HairF ($\beta = .074$, $p = .010$, $d = .28$) and not associated with the ratio HairF/HairE ($\beta = 0.054$, $p = .066$, $d = .27$). No other associations between HairF, HairE, the ratio HairF/HairE and use of antidepressants reached significance (all $p > .10$). The use of SSRIs and the severity of depression were independently associated with elevated HairF (use of SSRIs: $\beta = .070$, $p = .015$; severity depression: $\beta = .062$, $p = .038$) when analyzed within one model.

4. Discussion

The current study examined HairF and HairE levels as well as the ratio of HairF/HairE in a large sample with and without depression and anxiety. One aim of our study was to gain insight into the amounts of

Table 2
Associations between depressive and/or anxiety diagnosis status and HairF and HairE.

	HairF : model 1	HairF: model 2	HairE: model 1	HairE: model 2	Ratio: model 1	Ratio: model 2
	β	β	β	β	β	β
<i>Long-term glucocorticoids in relation to remitted and current diagnoses</i>						
Healthy controls (N = 245)	ref	ref	ref	ref	ref	ref
Any remitted depressive/anxiety disorder (N = 655)	< 0.001	0.003	−0.006	−0.002	0.030	0.029
Any current depressive/anxiety disorder (N = 266)	0.043	0.046	0.049	0.044	0.027	0.025
<i>Long-term glucocorticoids and current diagnoses</i>						
Healthy controls	ref	ref	ref	ref	ref	ref
Current depressive disorder (N = 78)	0.018	0.022	0.017	0.020	0.023	0.024
Current anxiety disorder (N = 122)	0.002	0.008	0.026	0.033	−0.019	−0.019
Current comorbid MDD/Anx disorder (N = 66)	0.072*	0.067*	0.033	0.032	0.059	0.055
<i>Long-term glucocorticoids and severity</i>						
IDS	0.064*	0.063*	0.015	0.018	0.057	0.054
BAI	0.054	0.052	0.019	0.023	0.056	0.052
FQ	0.049	0.040	0.005	0.001	0.062*	0.057
CTQ	0.043	0.038	0.043	0.040	−0.001	−0.002
<i>Long-term glucocorticoids and antidepressant medication</i>						
No medication	ref	ref	ref	ref	ref	ref
TCA	−0.025	−0.022	−0.027	−0.023	−0.010	−0.011
SSRI	0.072*	0.074*	0.040	0.046	0.058*	0.054
Other AD	−0.029	−0.029	−0.034	−0.037	−0.015	−0.012

Data are presented as standardized coefficients (β). *, $p < .05$; IDS, Inventory of Depressive Symptomatology; BAI, Beck Anxiety Inventory; FQ, Fear Questionnaire; CTQ, Childhood Trauma Questionnaire; TCA, Tricyclic Antidepressant; SSRI, Selective Serotonin Reuptake Inhibitor; AD, antidepressants. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, diagnosis of diabetes mellitus, hair washing frequency, season, and hair treatment.

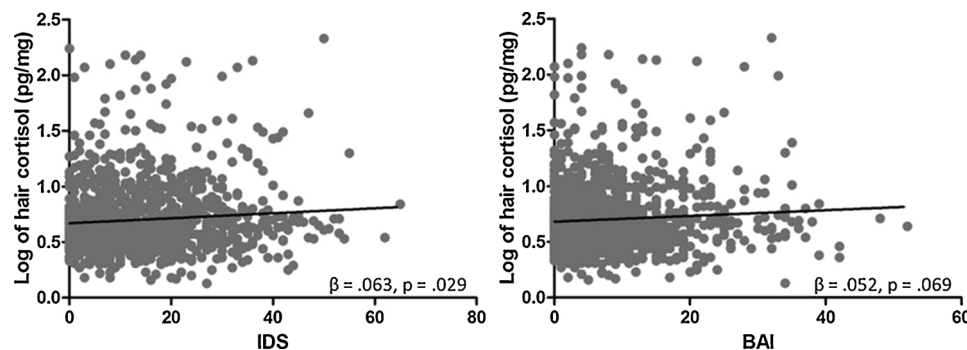


Fig. 2. Log-transformed HairF values and their association with the severity indexes for depression and anxiety. IDS, Inventory of Depressive Symptomatology; BAI, Beck Anxiety Inventory. Values are derived from fully-adjusted models.

long-term cortisol and cortisone, as well as their ratio, in depressive and anxiety disorders. We found that the comorbid MDD/Anx group had significantly higher long-term HairF levels than healthy controls and patients with a specific current or remitted diagnosis. But we did not observe any differences between healthy controls and patients with only a depressive or an anxiety disorder. One explanation for this result could be that the comorbid MDD/Anx group also experienced higher disease burden and daily impairments, and therefore increased stress that may come with a more severe disorder. Indeed, the comorbid MDD/Anx group had significantly higher scores on the depressive and anxiety severity scales than the other participants (as shown in Table 1). This hypothesis is supported by research showing that increased stress has often been associated with increased HairF (Russell et al., 2012; Stalder et al., 2014; Staufenbiel et al., 2013). A more technical explanation could be that the severity data had higher variation and thus more statistical power compared to analyses on the diagnostic groups. For MDD, conflicting results regarding the association between diagnosis and HairF have been reported (Lucia Dettenborn et al., 2012a,b; Hinkelmann et al., 2013; Pochigaeva et al., 2017; Stalder et al., 2014; Wei et al., 2015). Thus possibly, like in our study, only in the more severe cases higher hair cortisol levels can be

found.

We also explored whether subtypes of anxiety disorders were differently associated with HairF, HairE, or HairF/HairE. However, these results have to be interpreted cautiously due to the small sample sizes. We found indications that the diagnosis of social phobia may be associated with increased HairE, but not with HairF levels. One preceding study also reported no differences in HairF between healthy controls and patients with social phobia (Klumbies et al., 2014). We did not find associations of HairF, HairE, or HairF/HairE with any other anxiety disorder. For GAD inconsistent findings have been reported with either lower levels or no changes in levels (Steudte et al., 2011; Steudte-Schmiedgen et al., 2017) and one study on bipolar patients found lower HairF for patients with a comorbid panic disorder (Manenschijs et al., 2012). Thus so far, no consistent picture arises when examining hair cortisol levels and subtypes of anxiety disorders. Either this may mean that there are no differences between the subtypes, as in our study, or that bigger study samples are needed to show the (small) differences on cortisol levels between the separate anxiety subtypes.

Another aim was to use the information on the level of HairF, HairE, and the ratio HairF/HairE to gain new insights into the underlying pathophysiology of the HPA axis and its role in depressive and anxiety

disorders. This association seems to be less straightforward than initially assumed. The significant results were found in participants with a current (comorbid) diagnosis, whereas participants in remission did not show altered long-term glucocorticoid levels. This is an interesting result, as it poses a possible answer to the question whether a dysregulated HPA axis in mentally ill patients is a state or trait phenomenon. We found a stronger association with active cortisol rather than inactivated cortisol (cortisone), which is an interesting observation. It could be speculated that the alterations in HairF associated with psychopathological characteristics seem to be a state rather than a trait. This would imply that during the episode of a disorder, the HPA axis is dysregulated, but that the cortisol levels return to normal once the patient remits. Interestingly, a study that investigated differences in HairF between healthy controls, patients with a recurrent depressive disorder and patients with a first-episode disorder found that only patients with a first episode had increased HairF, whereas patients with a recurrent depressive episode had comparable HairF as healthy controls (Wei et al., 2015). The authors hypothesize that the duration or the number of recurring episodes of the depressive episode might alter the sensitivity of HPA axis of the patients with a recurrent depressive episode. This gives rise to the question whether the underlying pathophysiology is different for persons with a first/single episode compared to persons with recurrent episodes. In our study, the duration of mental disorders showed no association with long-term glucocorticoid levels. Long-term activation of the HPA axis can however also lead to down-regulation, resulting in lower cortisol levels (Ostrander et al., 2006). As such it could also be that the comparable levels in patients with recurrent episodes do not reflect return to normal levels, but a transition into hypo-activity of the HPA axis. Future research will have to further shed light on these associations.

Another interesting finding is that the use of SSRIs is associated with higher HairF. The use of antidepressants has been shown to influence the HPA axis in many ways, from regulation of glucocorticoid receptor (GR) expression to post-translational modifications, which may result in differences in GR nuclear translocation and GR-dependent gene transcription (Anacker et al., 2011; Barden, 2004). Two other studies reported associations between HairF and psychotropic drugs (Staufenbiel et al., 2014; Wells et al., 2014), whereas a third study did not find differences in HairF between users and non-users (Van Uum et al., 2008). Apart from the working mechanisms of SSRIs on the HPA axis, another explanation could be that patients with more severe depressive symptoms may tend to use medication as opposed to patients with minor symptoms. However, the use of SSRIs and the severity of depression were independently associated with elevated HairF, rendering this hypothesis unlikely. There could also have been some confounding by indication, because it has been shown that particularly patients presenting with a specific emotional symptom including anxiety benefit more from SSRI's than patients presenting with more atypical symptom clusters (Chekroud et al 2017). This could partly explain why we found an association between HairF and SSRI use.

For significant effects, effect sizes were calculated, with effects ranging from 0.24 to 0.53 and thereby reflecting small to moderate effects. This is in line with previously calculated effect sizes in long-term glucocorticoid research (Staufenbiel et al., 2013) in which for long-term cortisol and mental illness, most effect sizes were small to medium. Our effect sizes are also comparable to those of a study assessing salivary cortisol levels, in which persons with a comorbid anxiety and depressive disorder had higher morning cortisol levels than healthy controls, with effect sizes ranging from 0.25 to 0.30 (Vreeburg et al., 2010).

This study has several strengths and limitations that need to be acknowledged. The current study is the first study that included participants from a large, longitudinal cohort study on depression and anxiety. This allowed us to additionally study associations between long-term glucocorticoids and pure diagnostic subgroups free from other comorbid psychological disorders while still retaining sample sizes

comparable to other studies. Furthermore, extensive information on covariates and psychological characteristics were available. However, the cross-sectional nature of the study did not allow us to draw causal conclusions about the direction of associations between long-term glucocorticoids and depressive as well as anxiety symptoms. Another limitation is that although our study comprised a large sample size, the size of the diagnostic (sub)groups was rather small for some subgroups. Another factor that needs to be mentioned is that the time frame represented by the hair (i.e. three months) did not correspond to the time frame of the diagnosis used here (i.e. one month). This unequal time frame was caused by practical reasons, in that we had too little hair samples per person to allow for hair measurements of one cm. However, therefore we cannot exclude the possibility that we underestimate the effects of depressive and anxiety disorders on HairF and HairE, as also a healthy/diagnosis-free period may be covered by the hair sample.

In order for hair cortisol to become a true biomarker in the field of psychiatry we obviously need good clinical reference ranges that define when a level is within normal biologic range and when it is in a pathological range. So far we do not have these reference values yet for depressive and anxiety disorders. Given the small to moderate effect sizes observed in ours and other studies examining cortisol levels in psychiatric populations, it seems unlikely that with cortisol alone we will be able to set up a reliable and specific diagnostic or prognostic test. However, in combination with other allostatic load markers (Seeman et al 2007), such as inflammatory markers, glucose and nor-adrenalin this may be possible in the future.

5. Conclusion

To conclude, this study demonstrates that persons with comorbid anxiety and MDD diagnoses and current severe symptoms of depression or anxiety show higher long-term levels of cortisol than patients in remission and healthy controls. Given that we only found significant differences in the group with current and severe symptoms our results suggests that hair cortisol can be considered a biomarker for state rather than trait of depressive and anxiety disorders.

Conflict of interest statement

None of the authors have any conflict of interest to report. None of the funding sources had any involvement in the design, analysis, writing of this study.

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