Psychoneuroendocrinology (2015) 59, 91—101

Available online at www.sciencedirect.com

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journal homepage: www.elsevier.com/locate/psyneuen

DHEAS and cortisol/DHEAS-ratio in recurrent depression: State, or trait predicting 10-year recurrence?

R.J.T. Mocking[a,*], C.M. Pellikaan[a], A. Lok[a,b], J. Assies[a], H.G. Ruhé[a,c], M.W. Koeter[a], I. Visser[a], C.L. Bockting[d,e], M. Olff[b,f], A.H. Schene[a,g,h]

[a] *Program for Mood disorders, Department of Psychiatry, Academic Medical Center, University of Amsterdam, The Netherlands*
[b] *Arq Psychotrauma Expert Group, Diemen, The Netherlands*
[c] *University Medical Center Groningen, Program for Mood and Anxiety Disorders, Department of Psychiatry, The Netherlands*
[d] *Department of Clinical Psychology, University of Groningen, The Netherlands*
[e] *Department of Clinical and Health Psychology, Utrecht University, The Netherlands*
[f] *Center for Psychological Trauma, Department of Psychiatry, Academic Medical Center, University of Amsterdam, The Netherlands*
[g] *Department of Psychiatry, Radboud University Medical Center, Nijmegen, The Netherlands*
[h] *Donders Institute for Brain, Cognition and Behavior, Radboud University, Nijmegen, The Netherlands*

Received 17 February 2015; received in revised form 12 May 2015; accepted 12 May 2015

KEYWORDS
Hypothalamic—pituitary—adrenal (HPA) axis;
Major depressive disorder (MDD);
Dehydroepiandrosterone-sulphate (DHEAS);
Cortisol;
Remission;
Recurrence

Summary
Background: Major depressive disorder (MDD) has been associated with low dehydroepiandrosterone-sulphate (DHEAS), — particularly relative to high cortisol — although conflicting findings exist. Moreover, it is unclear whether low DHEAS is only present during the depressive state, or manifests as a trait that may reflect vulnerability for recurrence. Therefore, we longitudinally tested whether low DHEAS and high cortisol/DHEAS-ratio in recurrent MDD (I) reflects a trait, and/or (II) varies with depressive state. In addition, we tested associations with (III) previous MDD-episodes, (IV) prospective recurrence, and (V) effects of cognitive therapy.
Methods: At study-entry, we cross-sectionally compared morning and evening salivary DHEAS and molar cortisol/DHEAS-ratio of 187 remitted recurrent MDD-patients with 72 matched controls. Subsequently, patients participated in an 8-week randomized controlled cognitive therapy trial. We repeated salivary measures after 3 months and 2 years. We measured clinical symptoms during a 10-year follow-up.

* Corresponding author at: Department of Psychiatry, Academic Medical Centre, Meibergdreef 5, Amsterdam 1105 AZ, The Netherlands. Tel.: +31 208913695.
E-mail address: R.J.Mocking@amc.uva.nl (R.J.T. Mocking).

http://dx.doi.org/10.1016/j.psyneuen.2015.05.006

Results: Remitted patients showed steeper diurnal DHEAS-decline ($p < .005$) and a flatter diurnal profile of cortisol/DHEAS-ratio ($p < .001$) than controls. We found no state-effect in DHEAS or cortisol/DHEAS-ratio throughout follow-up and no association with number of previous episodes. Higher morning cortisol/DHEAS-ratio predicted shorter time till recurrence over the 10-year follow-up in interaction with the effects of cognitive therapy ($p < .05$). Finally, cognitive therapy did not influence DHEAS or cortisol/DHEAS-ratio.
Conclusions: Diurnal profiles of DHEAS and cortisol/DHEAS-ratio remain equally altered in between depressive episodes, and may predict future recurrence. This suggests they represent an endophenotypic vulnerability trait rather than a state-effect, which provides a new road to understand recurrent depression and its prevention.
Trial registration: www.isrctn.com/ISRCTN68246470.

1. Introduction

Major depressive disorder (MDD) represents a large burden of disease, mainly due to its high recurrence and cardiovascular comorbidity risks (Assies et al., 2014; Bockting et al., 2005). Indicatively, 80% of recovered MDD-patients experience an average of five recurrences during lifetime (Bhagwagar and Cowen, 2008), and cardiovascular disease is a leading cause of death in MDD (Assies et al., 2014). If we better understand recurrent MDD's pathophysiology, we may improve prevention of recurrence and cardiovascular disease in at-risk patients.

An important pathophysiological characteristic of MDD is altered activity of the hypothalamic—pituitary—adrenal (HPA)-axis (Stetler and Miller, 2011). HPA-axis hormone cortisol has been extensively studied, and mainly found to be present in higher concentrations in MDD-patients (Stetler and Miller, 2011), which was also reported by our group in the present study's sample of patients with recurrent MDD (Lok et al., 2012). The potentially detrimental effects of chronic high cortisol has been termed allostatic load (McEwen, 2007), which may explain e.g. reduced hippocampal volumes and atherosclerosis, contributing to the extensive recurrence and cardiovascular comorbidity rates in MDD.

However, more abundantly than cortisol, adrenal glands also secrete the neuroactive steroids dehydroepiandrosterone (DHEA) and its sulfate ester DHEAS [jointly referred to as DHEA(S)] (Maninger et al., 2009). While DHEA(S)' precise role remains unclear, DHEA(S) is thought to counteract cortisol's effects on allostatic load (Maninger et al., 2009). Specifically, while cortisol has a catabolic function, DHEA(S) seems to have an anabolic, regenerative, and neuroprotective function in the brain and cardiovascular system (Maninger et al., 2009), which may be mediated through effects on brain derived neurotrophic factor (BDNF) and gamma-aminobutyric-acid (GABA)-metabolism (Genud et al., 2009; Sakr et al., 2014). Consequently, the cortisol/DHEA(S)-ratio is proposed to represent a balance between catabolic and anabolic activity (Chen et al., 2015; Lennartsson et al., 2013).

Although DHEA(S) derived far less attention in MDD compared to cortisol, an elevated cortisol/DHEAS-ratio has been found in MDD-patients and has been proposed as a state marker of MDD (Maninger et al., 2009; Young et al., 2002). However, opposite results of no differences or higher DHEA(S) have also been reported (Assies et al., 2004; Maninger et al., 2009). These conflicting findings may be caused by the relatively small size and large heterogeneity of the investigated samples thus far (Maninger et al., 2009). Nevertheless, given DHEA(S)' anabolic effects (e.g. neuroprotection and regeneration), low DHEA(S) — particularly relative to cortisol — could be of clinical importance because it may intensify allostatic load and thereby contribute to recurrence and cardiovascular comorbidity in MDD (Juster et al., 2010). Therefore, assessment of DHEA(S) in addition to cortisol provides a more complete indication of HPA-axis functioning.

However, the precise characteristics of altered DHEA(S) in MDD remain unknown (Maninger et al., 2009). Using the sample of the present study, we previously suggested that high cortisol in MDD is a trait (indicating an endophenotype), not a state (epiphenomenon) (Lok et al., 2012). Whether this also holds true for DHEA(S) remains unknown, because of a lack of prospective repeated measures studies (Maninger et al., 2009). In addition, we observed that cortisol was relatively lower (suggesting HPA-axis blunting/exhaustion) in patients with more previous MDD-episodes (MDEs) (Lok et al., 2012). Other analyses in the recurrent MDD sample showed that cortisol predicted time to recurrence in interaction with cognitive therapy. In detail, while in remitted patients who did not receive cognitive therapy *lower* cortisol was associated with early recurrence; in patients who received cognitive therapy *higher* cortisol levels relatively predicted early recurrence (Bockting et al., 2012, 2006). Finally, we (Lok et al., 2012; based on analyses in the present study's sample) and others (Hsiao et al., 2011) found that psychotherapy resulted in steeper declines in diurnal cortisol. To the best of our knowledge, relations of these factors with DHEA(S) in MDD remain unclear.

Therefore, after examining the above relations in recurrent MDD for cortisol, we aimed to test the following hypotheses for DHEAS and cortisol/DHEAS ratio as well: (I) during remission DHEAS will be lower, and cortisol/DHEAS-ratio higher, than in never-depressed controls (suggesting a trait), (II) DHEAS or cortisol/DHEAS-ratio will not change during the depressive state, (III) more previous MDEs will be associated with lower DHEAS and a higher cortisol/DHEAS-ratio, (IV) higher DHEAS and lower ratio will predict longer time till prospective MDD-recurrence, and (V) psychotherapy will increase DHEAS and decrease the cortisol/DHEAS-ratio.

2. Methods and materials

2.1. Design

As described previously, for the current study we used a two-staged case—control and prospective-cohort design, that was integrated in a randomized controlled trial assessing recurrence preventing effects of cognitive psychotherapy in recurrent MDD (Bockting et al., 2005, 2006, 2012; Lok et al., 2012). First, in the case—control stage, we obtained saliva samples at study-entry (T0) to cross-sectionally compare patients with controls. Subsequently, we randomized patients to treatment as usual or an additional preventive cognitive therapy (CT)-module. This module consisted of eight weekly group sessions focussing on identification and change of dysfunctional attitudes (Bockting et al., 2005). Treatment as usual consisted of naturalistic care, ranging from continuous antidepressant treatment to no treatment at all. After the 8-week intervention period, we repeated saliva sampling after 3 months (T1) and 2 years (T2) in the patients (Lok et al., 2012), and performed a 10-year follow-up of clinical symptoms.

2.2. Study sample

After approval by the ethics committee of the Academic Medical Center of the University of Amsterdam, we recruited 18—65 years old MDD-patients with ≥2 previous MDEs in the last 5 years according to the structural clinical interview for DSM-IV disorder (SCID) which reached remission 10 weeks to 2 years ago, defined as a score ≤9 on the 17-item Hamilton Depression Rating Scale (HDRS) (Lok et al., 2012). We excluded patients with a history of any psychotic, bipolar, or predominant anxiety disorder, organic brain damage, alcohol/drug abuse/dependency, or current steroid use. All subjects gave informed consent. We recruited controls matched for sex and age with no current/past (personal and/or family) axis-I disorders, assessed with the SCID, and no current steroid use.

2.3. Study measures

2.3.1. Depression characteristics and covariates

At T0, we measured educational level (low, middle, and high), anthropometric measures (body mass index, waist and hip circumference), smoking behavior and medication use (including contraceptives) for both patients and controls.

In addition, in patients, we measured current and past MDEs at T0, and at 11 follow-up measurements (at T1, every 3 months until T2, and after 36, 66, and 120 months, i.e. 10 years) using the SCID. In line with previous reports on the present study's sample (Bockting et al., 2005; Lok et al., 2012), we operationalized previous MDEs dichotomously using a median split (<5 or ≥5 MDEs), because of severe violation of the normality assumption. We address both relapses (<6 months after a previous MDE) and recurrences as 'recurrence' for clarity reasons. The trained SCID-evaluators were blind to treatment condition; subjects were instructed not to reveal treatment condition to the interviewers (psychologists/research assistants). We audio-taped all interviews, and two independent experienced psychiatrists — blinded to treatment condition — evaluated all occasions of participants meeting DSM-IV criteria for an MDE. In case of disagreement, psychiatrists' ratings were used. Kappa for inter-rater agreement between interviewers and psychiatrist on categorization of a relapse/recurrence or no relapse/recurrence was .96, indicating high agreement (Bockting et al., 2005; Lok et al., 2012).

2.3.2. Hormone measures

In the saliva samples obtained at T0 in both patients and controls, and additionally at T1 and T2 in patients, we measured cortisol and DHEAS. At each of these three time points, we collected three saliva samples using neutral cotton salivettes (Sarstedt™): day 1 at 0800 h, day 1 at 2200 h, and day 2 at 0800 h. Salivettes provide a relatively stress free way to obtain hormone measures; they are also applicable for DHEAS (Whetzel and Klein, 2010). As described previously in our report on the cortisol measures of the present study's sample (Lok et al., 2012), we instructed participants not to eat overnight, not to brush their teeth and rinse their mouth with water immediately before collecting their saliva, to keep their samples refrigerated, and to send them back by mail on the second day. After receipt, we stored samples at −20 °C until radioimmunoassay analysis (IBL, Hamburg; designed for saliva samples). Intra- and interassay variations were 5.1% and 6.5% for cortisol, and 7.3 and 7.9% for DHEAS, respectively.

2.4. Data analysis

2.4.1. Data cleaning and multiple imputation

We considered measures exceeding >4 standard deviations from the mean as missing, because of suggestive blood contamination (Lok et al., 2012). To reduce bias potentially introduced by missing values, we applied multiple imputation using Amelia II to obtain less biased effects estimates (Donders et al., 2006; Lok et al., 2012). We used five separate imputed data sets for both our study designs (i.e., cross-sectional and longitudinal prospective cohort-study) (Lok et al., 2012). After imputation, we tested whether the day-to-day variability in the two subsequent morning DHEAS measurements was comparable between patients and controls, so we could take the average. The within-subject coefficient of variation for the two subsequent morning T0 measurements was 31.67%, and did not differ between patients and controls ($P = .480$) (Bland and Altman, 1996). Therefore, we calculated mean DHEAS over the 2-day morning measurements at T0, T1, and T2. In addition, we calculated the molar cortisol/DHEAS-ratio by dividing cortisol by DHEAS molar concentrations. We used natural log transformations to obtain normal distributions. To get pooled test results from the imputed data sets, we used an SPSS-macro (Lok et al., 2012).

2.4.2. Subject characteristics and propensity scores

We compared baseline characteristics of patients and controls using Chi-square and Student's independent *t*-test statistics. We used a propensity score to adjust for multiple potential confounders without losing too much statistical power. We calculated a propensity score (PS1) for the

cross-sectional analyses in patients and controls, based on sex, age, educational level, contraceptive use, last month steroid use, smoking, weight and waist and hip circumference. For the longitudinal analyses (except for the effect of randomized CT) in patients we calculated another propensity score (PS2) additionally including alcohol and drug use during follow-up (yes/no), benzodiazepine use (yes/no), treatment with CT (yes/no) and continuous use of antidepressants (yes/no) (Lok et al., 2012).

2.5. Statistical analyses

We used SPSS Statistics 20.0 (IBM Corp., 2011) for all statistical analyses. In accordance with our previous report on cortisol measures from the present study's sample (Lok et al., 2012), we tested hypotheses I—III and V using marginal linear regression models with unstructured covariance matrices with DHEAS or cortisol/DHEAS-ratio as the dependent variable. For hypothesis I, independent variables were sampling moment (morning/evening), group (patient/control) and the moment × group-interaction. For the subsequent hypotheses, independent variables were follow-up time (T0/T1/T2), sampling moment (morning/evening) and the appropriate covariate for each research questions, i.e. depressive state as a time-dependent factor at T0, T1 and T2 (yes/no) for hypothesis II; previous MDEs determined at T0 (<5 or ≥5 MDEs) for hypothesis III; and CT (yes/no) for hypothesis V, including relevant interactions. When higher order interactions were not significant we removed them from the model and used the most parsimonious model. For our fourth hypothesis (do T0 hormone concentrations predict time until recurrence?) we used a Cox proportional hazard model. Because we previously showed an interaction between previous MDEs and CT (Bockting et al., 2005), we used DHEAS or cortisol/DHEAS-ratio, treatment condition (treatment as usual vs. CT), previous MDEs (≥5 vs. <5) and their interactions as predictors, and time till the start of the first recurrence or end of observation during the 10-year follow-up in days as right censored dependent variable. As Cox-models cannot combine these, we used separate Cox models for morning and evening measures.

3. Results

3.1. Subject inclusion, characteristics, and missing data (Table 1)

During inclusion, approximately 1000 subjects (31% recruited at psychiatric centers; 69% through media announcements) were telephonically screened, 321 were interviewed, resulting in 187 included patients. In addition, 72 matched controls were included. Of the patients, 15 dropped out of the study's CT, but HPA-axis data was collected so they were included in all analyses. Drop-outs were younger than completers, but did not differ on other characteristics ($p > .05$). For the 172 remaining patients 10.7%, 21.7% and 42.6% of the hormone measures was missing at T0, T1 and T2 respectively. For the 72 controls 10.6% was missing at T0. In total 1361 measures were obtained at T0, T1 and T2, from which seven were assigned missing due to probable blood contamination.

Patients and controls were successfully matched on sex and age; however, patients had a lower educational level, higher weight and larger waist circumference (Table 1). Patients had a mean of 6.3 previous MDEs. During the 10-years follow-up an estimated 82.4% (154/187) experienced a recurrence.

3.2. Hypothesis I: differences between remitted MDD-patients and controls (Fig. 1)

The diurnal course of DHEAS over the day showed a significantly steeper decline in patients than in controls (group × moment interaction; $p = .001$). These effects remained after adjustment for potential confounders using PS1. Post hoc tests comparing morning and evening values separately, showed that morning DHEAS did not differ, while evening DHEAS was significantly lower in patients compared to controls ($p = .001$).

For the cortisol/DHEAS-ratio, the patients showed a flatter diurnal profile than controls (group × moment interaction; $p < .001$), which remained after correction for confounders. Post hoc tests showed no differences in the morning, but a significantly higher ratio for patients compared to controls in the evening ($p < .001$).

3.3. Hypothesis II: changes during an MDE (Fig. 2)

In patients, DHEAS and cortisol/DHEAS-ratio were not associated with the state of being depressed (yes/no) during follow-up according to the SCID at the given sampling timepoints (T0/T1/T2) (state-effect; $p = .566$, $p = .330$, respectively), also not after omitting the equally non-significant effect of course over the day (state × moment-interaction; $p = .941$, $p = .617$, respectively).

3.4. Hypothesis III: association with previous MDEs (Supplemental Fig. 1)

There were no significant differences between patients with ≥ 5 previous MDEs compared to patients with <5 previous MDEs for DHEAS and cortisol/DHEAS-ratio (previous MDEs-effect, $p = .743$ and $p = .803$, respectively), their courses over the day (MDEs × moment-interaction; $p = .686$ and $p = .569$) and follow-up (MDEs × follow-up-interaction; $p = .527$ and $p = .617$). Adjustment for confounders did not change these findings.

Supplementary Fig. 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.05.006.

3.5. Hypothesis IV: association with recurrence (Fig. 3)

The analysis of the predictive effect of study-entry (T0) hormone concentrations on prospective time until recurrence over the 10-year follow-up revealed a significant three-way interaction between morning cortisol/DHEAS-ratio, previous MDEs and treatment condition (CT vs. treatment as usual;

Table 1 Subject characteristics.

Characteristic	Patients ($N = 187$)	Controls ($N = 72$)	p value
Female %	68.1	72.7	.456
Age, mean (SD), year	44.2 (9.7)	44.9 (9.3)	.61
Educational level			<.001
Low, %	33.2	4.6	
Middle, %	32.6	19.7	
High, %	34.2	72.3	
Smoking, %	29.9	22.9	.28
Weight, mean (SD), cm	78.9 (16.3)	73.76 (13.4)	.04
Waist circumference, mean (SD), cm	89.3 (13.9)	83.7 (12.3)	.01
Hip circumference., mean (SD), cm	105.3 (11.1)	103.1 (7.8)	.13
Oral contraceptive use, %	22.1	17.1	.40
Steroid use in month before assessment, %	.6	1.4	.57
Benzodiazepine use, %	8.0	NA	
Continuous AD use during follow-up, %	27.3	NA	
Antidepressant use at study entry, %	42.2	NA	
TCA, %	3.9	NA	
SSRI, %	29.2	NA	
Other, %	9.1	NA	
Received cognitive therapy, %	51.9	NA	
$HDRS_{17}$, score, mean (SD)	3.8 (2.9)	NA	
Number of previous episodes, mean (SD)	6.3 (8.1)	NA	
Five or more previous episodes, %	40.6	NA	
Age of onset first episode, mean (SD), year	28.5 (12.5)	NA	
Depressed at T1, %	15.0	NA	
Depressed at T2, %	16.0	NA	

Abbreviations: AD, antidepressant; HDRS, Hamilton depression rating scale; SSRI, selective serotonin reuptake inhibitor; T0, study-entry; T1, T2, 3 months and 2 years of follow-up respectively; TCA, tricyclic antidepressant.

Wald statistic$_{1,N=187} = 5.08$, $p = .026$, hazard ratio = .48, 95% CI = 0.25—0.92). The evening cortisol/DHEAS-ratio showed a similar trend (Wald statistic$_{1,N=187} = 2.88$, $p = .091$, hazard ratio = .60, 95% CI = 0.33—1.09), in line with an opposite trend for morning DHEAS itself (Wald statistic$_{1,N=187} = 3.22$, $p = .073$, hazard ratio = 1.83, 95% CI = 0.94—3.55). The three-way interaction for evening DHEAS also showed an opposite but non-significant effect (Wald statistic$_{1,N=187} = 2.21$,

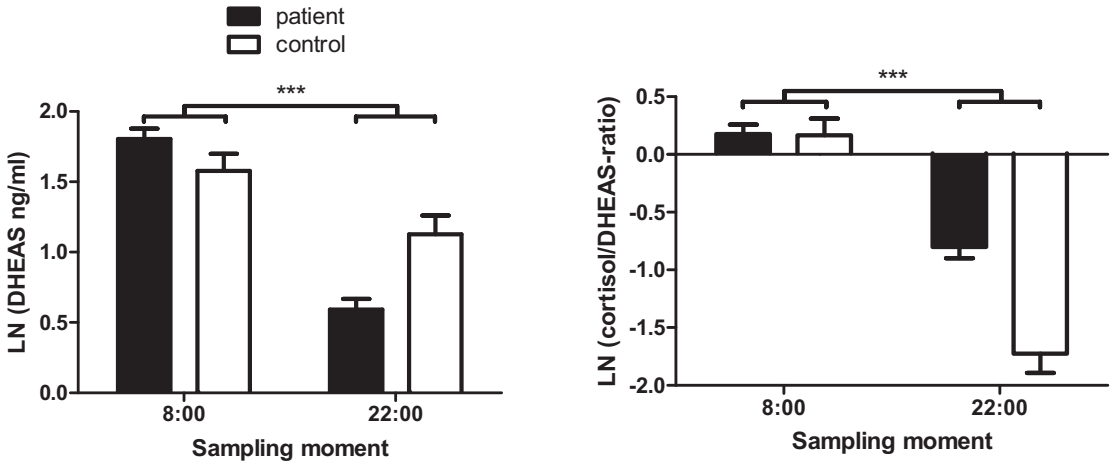

Figure 1 DHEAS concentrations and cortisol/DHEAS-ratio for recurrent MDD-patients in remission ($N = 187$) compared to controls ($N = 72$). Compared to matched controls, patients showed a steeper decline in DHEAS' diurnal course over the day and a flatter diurnal profile of molar cortisol/DHEAS ratio. Marginal linear regression model analyses results; remitted patients versus controls group × moment interaction $F_{1,441.59} = 11.26$, $p = .001$; $F_{1,98,33} = 20.24$, $p < .001$, for DHEAS and ratio, respectively. Results were adjusted for sex, age, educational level, contraceptive and steroid use, smoking, weight and waist and hip circumference. Error bars indicate SE, *** indicates $p \leq .001$.

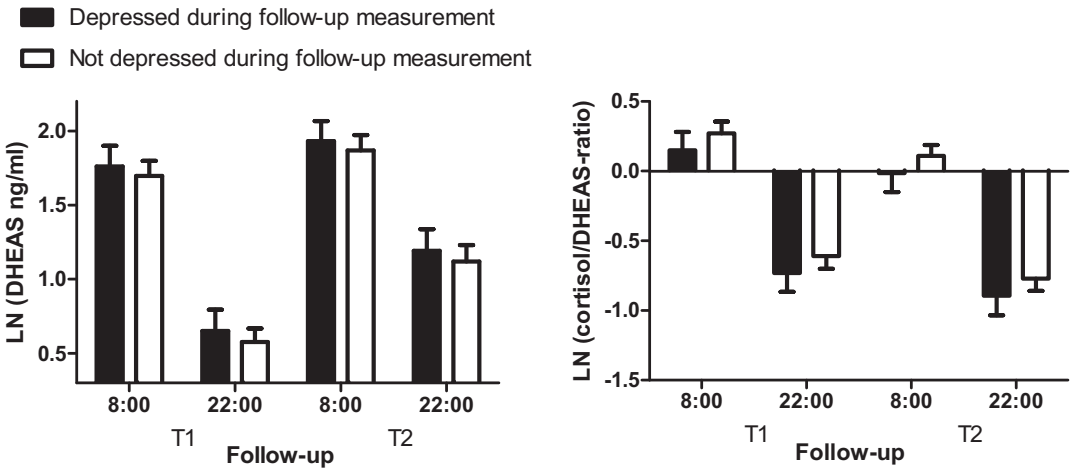

Figure 2 DHEAS concentrations and cortisol/DHEAS-ratio for patients that had a current depressive episode compared to patients that did not experience a recurrence during sampling moment T1 (after 3 months) and T2 (after 2 years of follow up). Both DHEAS and molar cortisol/DHEAS ratio showed no changes during a follow-up recurrence. Marginal linear regression model analyses results for DHEAS and cortisol/DHEAS-ratio $F_{1,30.18} = .34$, $p = .566$; $F_{1,28.13} = .981$, $p = .330$, respectively. Error bars indicate SE. Measures obtained at study entry (T0) were not included in the figure because all MDD patients were in remission at T0 as according to the inclusion criteria. These measurements were included in the analysis though.

$p = .138$, hazard ratio = 1.57, 95% CI = 0.87—2.86). Lower order interactions and main effects were not significant.

Post hoc tests for the significant three-way interaction of the morning cortisol/DHEAS-ratio showed that it was driven by a two-way interaction of cortisol/DHEAS-ratio and CT in patients who had experienced <5 previous MDEs. More specifically: in patients with <5 previous MDEs, cortisol/DHEAS-ratio (dichotomized for graphical purposes, using median split) showed a significant two-way interaction with CT (Wald statistic$_{1,N=111} = 5.48$, $p = .021$, hazard ratio = .32, 95% CI = 0.12—0.84). In patients with <5 MDEs who were randomized to CT a higher ratio was associated with shorter time until recurrence, while in patients with <5 MDEs who were randomized to treatment as usual a higher ratio

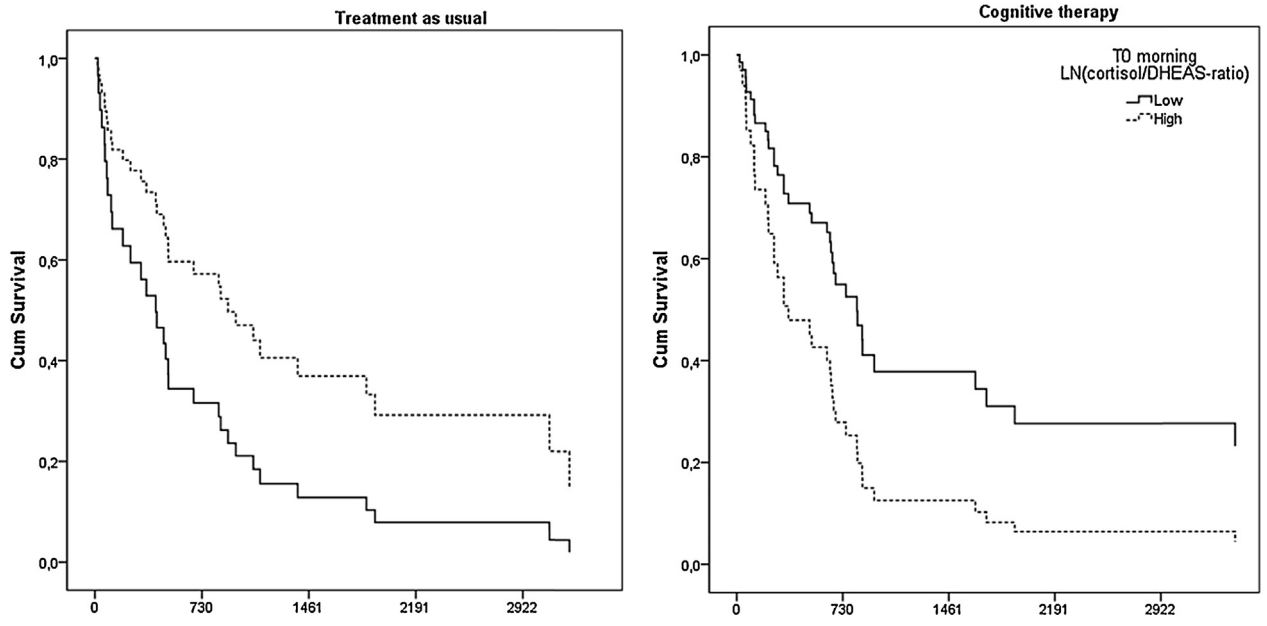

Figure 3 Graphical display of the post hoc analyses for the significant three-way interaction between morning cortisol/DHEAS-ratio, previous MDEs and treatment condition (CT vs. treatment as usual) that predicted time till recurrence over the 10-year follow-up in recurrent MDD patients with <5 previous MDEs. Specifically, in patients with <5 previous MDEs, dichotomized molar cortisol/DHEAS-ratio showed a significant two-way interaction with CT. In detail, in patients with <5 MDEs that were randomized to CT a higher ratio was associated with shorter time till recurrence (right panel), while in patients with <5 MDEs that were randomized to treatment as usual a higher ratio was associated with longer time till recurrence (left panel).

was associated with longer time till recurrence. This two-way interaction was not present in patients with ≥5 MDEs ($p = .686$).

3.6. Hypothesis V: effect of CT (Supplemental Fig. 2)

After eight weeks of CT, DHEAS and cortisol/DHEAS-ratio during follow-up at T1 and T2 of patients who received CT were compared with patients who had not received CT. Three-way, two-way and main effects of CT were not significant, indicating that CT had no overall effect on DHEAS or cortisol/DHEAS-ratio, nor their courses over the day or follow-up.

Supplementary Fig. 2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.05.006.

4. Discussion

The present study first cross-sectionally compared remitted MDD-patients with matched controls, and showed a steeper DHEAS-decline over the day and a flatter diurnal profile of cortisol/DHEAS-ratio in patients compared to controls. Particularly evening values were altered, with lower DHEAS and higher ratios in patients. Repeated measures during follow-up of patients showed no changes in DHEAS and cortisol/DHEAS-ratio during new MDEs. Furthermore, higher study-entry morning cortisol/DHEAS-ratios relatively predicted sooner recurrence during 10-years follow-up in patients that were randomized to CT and experienced <5 previous MDEs. Finally, CT did not influence DHEAS and cortisol/DHEAS-ratio.

4.1. Hypothesis I and II: steeper diurnal DHEAS decline and flatter diurnal profile of cortisol/DHEAS-ratio as a trait, not a state

4.1.1. Directions of alterations

Our results show that the direction of alterations depends on time of the day: DHEAS had a steeper decline over the day, while the cortisol/DHEAS-ratio diurnal profile was flatter compared to controls. Differences were most pronounced in the evening, i.e. lower DHEAS and higher ratio. This fits with the general findings in previous literature in MDD (Maninger et al., 2009), and with findings of higher cortisol in the present study's sample (Lok et al., 2012). However, contradictory findings exist (Assies et al., 2004; Heuser et al., 1998; Maninger et al., 2009). This heterogeneity may be explained by several factors, including patient characteristics (age, MDD-subpopulation, medication use), methodological factors (stress-free salivary vs. blood samples, sampling time, small sample sizes), or theoretical aspects (DHEA vs. DHEAS-measurements, using ratio over cortisol) (Maninger et al., 2009). Our study provides additional knowledge, by using repeated measures to show stable steeper diurnal DHEAS-declines and flatter diurnal cortisol/DHEAS-ratio profiles in saliva in a specific but large group of recurrent MDD-patients compared to carefully matched controls. This again stresses the importance of diurnal timing of measurements, and the difference of using DHEAS vs. its cortisol-ratio, which may guide future studies aimed at corroborating these findings to further unravel the precise role of DHEA(S) in MDD.

4.1.2. Trait vs. state

In accordance with our hypotheses, our longitudinal results show that the HPA-axis alterations do not change during an MDE, which suggests a trait nature. This is in line with results for only cortisol in the present study sample (Lok et al., 2012). Moreover, these findings correspond with earlier cross-sectional studies observing persistent DHEAS-alterations in a small sample of remitted MDD-patients (Girdler et al., 2012), and a state-independent trait of DHEAS-alterations in schizophrenia (Ritsner et al., 2007). However, it may seem inconsistent with cross-sectional studies showing DHEA(S)-differences in remitted compared to depressed MDD-subjects and associations between DHEAS and MDD-symptom severity (Michael et al., 2000). Moreover, previous studies showed associations between DHEA(S)-reductions and remission during antidepressant treatment (Fabian et al., 2001; Hsiao, 2006; Morita et al., 2014; Paslakis et al., 2010; Schule et al., 2009). However, other studies suggested that these associations between DHEA(S) and remission already exist before treatment (Goodyer et al., 2003; Kurita et al., 2013; Markopoulou et al., 2009) or even before MDD-onset (Goodyer et al., 2000).

In sum, a seemingly inconsistent picture arises: MDD-patients exhibit DHEA(S)-alterations (Maninger et al., 2009), that seem to be equally present before the first MDE (Goodyer et al., 2000) and in between subsequent MDEs (i.e. trait instead of state) [present data and (Girdler et al., 2012)]. On the other hand, DHEA(S) is cross-sectionally associated with MDD-symptom severity (Michael et al., 2000) and successful treatment with antidepressants (Morita et al., 2014; Paslakis et al., 2010; Schule et al., 2009). This seems to be similar to what have been demonstrated for cortisol (Lok et al., 2012); several explanations for these apparent inconsistencies could be thought of.

The associations between DHEAS and symptom severity reported in previous cross-sectional studies could be compatible with a trait when they actually reflect an indirect effect instead of a direct state-effect (Lok et al., 2012). For instance, patients who are most vulnerable to MDD could exhibit both a more outspoken trait of DHEAS-alterations and more MDD-symptoms at a given cross-sectional measurement point. Thereby, this indirect effect — next to other possible confounding factors — may explain the previously observed cross-sectional associations. We now present the current, to the best of our knowledge first, long-term longitudinal study, which enables us to disentangle these direct and indirect effects using a within-subject repeated measures design. This approach suggests that there are no changes in DHEAS when a remitted patient enters a new MDE.

Regarding the association between DHEA(S) and successful antidepressant treatment, it may be that the DHEA(S)-reductions do not reflect symptom change (i.e. a state effect), but are rather a proxy for (the possibility of) successful treatment (Ruhé et al., 2015). For example, as

noted before (Ruhé et al., 2015), antidepressants' effect on multidrug resistance p-glycoprotein may be a prerequisite for their clinical effectiveness (Pariante, 2008; Pariante et al., 2004). Given that DHEAS also is a substrate for p-glycoprotein (Bortfeld et al., 2006; Zelcer et al., 2003), the changes in DHEAS during successful antidepressant treatment may not reflect a state-effect, but rather the effective modulation of p-glycoprotein by the antidepressant (Ruhé et al., 2015).

Altogether, previous literature and the current study's data suggest that similar to findings for cortisol in the present study's sample (Lok et al., 2012), DHEAS-alterations form part of an endophenotypic HPA-axis trait in recurrent MDD (Hasler et al., 2004). However, inconsistencies regarding the direction of alterations and changes during antidepressant treatment still preclude firm conclusions (Maninger et al., 2009). Also given that the present study is the first long-term longitudinal repeated measures study of DHEAS in MDD, it is important to confirm results in future studies.

4.2. Hypothesis III: no association with previous MDEs

In contrast to our third hypothesis, we found no associations of DHEAS(-ratio) with number of previous MDEs. This suggests that there are no exhaustion or scarring effects of previous MDEs on DHEAS. This contrasts findings in present study's sample showing that cortisol alone was present in lower concentrations in patients with more previous episodes (Lok et al., 2012). This could also be interpreted as support for the notion that DHEAS-alterations might reflect a relatively stable endophenotype in recurrent MDD.

4.3. Hypothesis IV: predictive effect on time till recurrence

Our 10-year follow-up provided the opportunity to test predictive effects of DHEAS and cortisol/DHEAS-ratio on time till recurrence. Moreover, because the study was integrated in a randomized controlled trial, we could test interaction-effects with recurrence-preventing CT as we previously did for cortisol in the present study's sample (Bockting et al., 2005, 2006). Interestingly, we observed an interaction between morning cortisol/DHEAS-ratio and treatment in patients with <5 previous MDEs. Of note, the direction was as expected in patients randomized to CT (higher ratio predicted detrimental clinical course), but opposite in the treatment as usual group. Given the lack of previous studies investigating these predictive relations, interpretation remains somewhat speculative. The unexpected finding that *low* cortisol/DHEAS-ratio was associated with *shorter* time till recurrence in the treatment as usual group may correspond with earlier findings in the present study's sample showing that *higher* cortisol *protected* against recurrence over 5.5 years in the patients that did not receive CT (treatment as usual group) (Bockting et al., 2012). The effect was in the expected direction in the CT-group: *low* cortisol/DHEAS-ratio was associated with *longer* time till recurrence. This could be caused by enhancing effects of DHEAS on memory and cognition, which make patients with a relatively lower cortisol/DHEAS-ratio more receptive for CT's recurrence preventing effects (Maninger et al., 2009; Sripada et al., 2013). Analogically, lower cortisol (and higher DHEAS) prospectively predicted post-traumatic stress disorder (PTSD)-symptoms in trauma center patients (Mouthaan et al., 2014).

4.4. Hypothesis V: no effects of CT

Contrary to our hypotheses, our intention to treat analysis showed no effects of randomized CT on DHEAS or cortisol/DHEAS-ratio. This differs from findings [also in the present study's sample (Lok et al., 2012)] showing that CT can influence cortisol in MDD and PTSD (Hsiao et al., 2011; Olff et al., 2007). Nevertheless, this lack of effect of CT on DHEAS again contributes to the view of DHEAS-alterations as an endophenotype in MDD. In addition, it suggests that if CT's effects in MDD are (partly) mediated through the HPA-axis, they are not general but selective for cortisol.

4.5. Strengths and limitations

Some limitations should be noted. First, we did not obtain information on awakening time nor multiple consecutive morning saliva samples on the same day. Although it remains uncertain whether DHEAS shows an awakening response like cortisol (Clow et al., 2010), we consequently could not include this possible awakening response in our models. Second, we did not include detailed lifestyle variables in our model, e.g. physical activity, employment status, sleep pattern, and weekday vs. weekend sampling. However, we did correct for e.g. alcohol/drug use, smoking, weight, and waist/hip circumference, which are known to be associated with other lifestyle variables (Lok et al., 2012; Vreeburg et al., 2009). Possible consequences of these two shortcomings may be twofold, on the one hand they could have induced differences that do not directly reflect MDD's pathophysiology, e.g. when patients handled sampling protocols differently. On the other hand, these variables could have increased external variability thereby decreasing the ability to detect differences. Nonetheless, we observed consistent and relevant effects, systematically using identical methodology in patients and controls (Lok et al., 2012). Third, we did not distinguish between different MDD-subtypes, for which the cortisol/DHEAS-ratio may be an interesting addition in future research given the observed cortisol differences between e.g. melancholic and atypical MDD (Lamers et al., 2013). In addition, in order to specify the endophenotype, it may be interesting to investigate the association of DHEAS with a specific symptom of the depressive spectrum [e.g. libido loss, considering DHEA(S)' androgenic effects] instead of the whole heterogeneity of symptoms. Fourth, the present study was a naturalistic study, which implies that antidepressant use was no exclusion criterion. However, we observed no effect of antidepressant use in patients (continuous use yes/no) on cortisol or DHEAS in post hoc tests (p's > .554), and corrected for antidepressant use in the longitudinal analyses. Fifth, we did not include measures of stress or psychological trauma in our models. However, post hoc analyses showed no association of DHEAS with childhood life event- or daily hassle-questionnaire-scores (Lok et al.,

2012), neither during follow-up (p's > .511). Sixth, DHEAS' hydrophilic nature may reduce its passage into saliva, which has been suggested to limit the applicability of DHEAS as a salivary biomarker. However, evidence shows that (I) correlations between serum and saliva DHEAS-concentrations are comparable to those for cortisol, also after stress, and (II) salivettes and passive drool collection methods can be used interchangeably (Whetzel and Klein, 2008, 2010). Finally, we did not additionally measure DHEA. Although DHEAS can be converted into DHEA and vice versa, it would have been interesting to also measure DHEA to improve comparability with previous literature specifically on DHEA (Maninger et al., 2009).

Our study also had its strengths, especially our exclusive study sample and unique design. We included a large, relatively homogeneous, sample of patients with highly recurrent MDD (Lok et al., 2012). In addition, combining cross-sectional patient—control comparisons with repeated saliva samples over a long-term period, allowed us to specifically assess HPA-axis stability and disentangle trait- and state-effects. Finally, our very long-term 10-year follow-up enabled to interpret the observed HPA-axis alterations from a long-term clinical perspective.

4.6. Relevance and implications for research and clinic

Although the field moved on from the idea of DHEA(S) as a fountain of youth (Stewart, 2006), studies supplementing DHEA(S) for MDD or depressive symptoms suggest some promising effects (Peixoto et al., 2014). However, evidence is limited and long-term risks — including possible carcinogenic effects (Arnold, 2009; Key et al., 2002) — potentially limit clinical applicability. Investigation of 7-keto DHEA may be an interesting alternative (Bicanic et al., 2013; Davidson et al., 2000). However, our results show alterations that differ during the day but seem to remain stable over disease states, suggesting that timing of administration could be important, both diurnally and during disease progression.

Next to supplementation, our results suggest that DHEA(S) could also be used as a biomarker to predict clinically relevant events. For example, the observed DHEAS-trait can be part of an allostatic load biomarker panel (Juster et al., 2010), which may ultimately serve as a clinical tool to indicate patients that are at increased risk for recurrence and/or cardiovascular disease, guiding the clinician in his preventive treatment. In addition, knowing that patients with a lower cortisol/DHEAS-ratio may be more receptive to CT could help the clinician to better select the optimal recurrence preventing treatment for each individual patient, thereby providing a means to optimize precision/personalized treatment (Bockting et al., 2013).

5. Conclusion

Results show low evening DHEAS and high evening cortisol/DHEAS-ratio in recurrent MDD, that do not change during an MDE and were not influenced by previous MDEs or CT. This may suggest that, similar to earlier findings for cortisol in the present study's sample, DHEAS is part of an endophenotypic HPA-axis trait in recurrent MDD. In addition, the fact that the interaction between morning cortisol/DHEAS-ratio and CT-treatment predicted time till recurrence over a 10-year follow-up, may suggest that HPA-axis measures can be used to personalize preventive treatment in recurrent MDD.

Role of the funding sources

Funding support for this study was provided by the Netherlands Foundation for Mental Health, Utrecht and the Netherlands Organization for Health Research and Development Prevention Program (ZonMw). Both had no further role in study design; the collection, analysis and interpretation of data; the writing of the report; nor the decision to submit the paper for publication.

Conflict of interest

None declared.

Acknowledgements

We are most grateful to the participants of our study and we express our appreciation to the participating psychiatric sites for their recruitment efforts. We also thank our interviewers and independent raters and specifically Irene Visch for assistance with data management and support. The following colleagues also contributed to the DELTA (Depression Evaluation Longitudinal Therapy Assessment) Study: Mascha ten Doesschate, Jochanan Huyser, Guido Nabarro, Philip Spinhoven, Ellie Wekking and Luuk Wouters.

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