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Cortisol, dehydroepiandrosterone sulfate, fatty acids, and their relation in recurrent depression

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ABSTRACT

Background: Alterations in hypothalamic–pituitary–adrenal (HPA)-axis activity, fatty acid metabolism, and their relation have been associated with (recurrent) major depressive disorder (MDD), although conflicting findings exist.

Aims: To determine whether alterations in HPA-axis activity and fatty acids in recurrent MDD remain during remission (i.e. reflect a potential trait factor). Furthermore, to test the association between HPA-axis activity and fatty acids in patients versus controls.

Methods: We cross-sectionally compared 73 remitted unmedicated recurrent MDD patients with 46 matched never-depressed controls. Measurements included salivary cortisol and dehydroepiandrosterone sulfate (DHEAS) (awakening, evening, and after sad mood induction) and erythrocyte fatty acid parameters: (I) three main fatty acids [omega-3 docosahexaenoic acid (DHA), and the omega-3 eicosapentaenoic acid/omega-6 arachidonic acid (EPA/AA)-ratio], and (II) structural fatty acid indices [chain length, unsaturation and peroxidation].

Results: Patients showed higher cortisol awakening responses ($p = 0.006$) and lower evening cortisol/DHEAS ratios ($p = 0.044$) compared to matched controls. Fatty acids did not differ between patients and controls, but HPA-axis indicators were significantly associated with fatty acid parameters in both groups ($0.001 \leq p \leq 0.043$). Patients and controls significantly differed in the relations between awakening DHEAS or cortisol/DHEAS ratios and fatty acid parameters, including unsaturation and peroxidation indices ($0.001 \leq p \leq 0.034$). Significance remained after correction for confounders.

Conclusions: Our results further support alterations in HPA-axis activity, i.e. a lower baseline, but higher responsiveness of awakening cortisol, in remitted medication-free recurrent MDD patients. Furthermore, the relationship between HPA-axis and fatty acids showed significant differences in recurrent MDD patients versus controls. Prospective research is needed to determine the predictive value of this relationship for MDD recurrence.

1. Introduction

The overwhelming burden of major depressive disorder (MDD) is mainly due to its recurrent nature (Charlson et al., 2011). Suggested mechanisms underlying the recurrent course of MDD include alterations in endocrinology and metabolism.

From an endocrinological viewpoint, MDD has been extensively associated with hyperactivity of the principal stress system: the hypothalamic–pituitary–adrenal (HPA)-axis (Lok et al., 2012; Stetler and Miller, 2011). However, inconsistencies exist with some studies observing no HPA-axis dysregulation (Ruhé et al., 2015) or even HPA-axis hypoactivity (Ahrens et al., 2008; Bockting et al., 2012; Wichmann et al., 2017). Similarly, effects of sad mood on cortisol have been observed to differ between MDD patients and controls, suggesting a higher HPA-axis responsiveness to stressors in MDD (Huffziger et al., 2013), although a lower responsiveness has been reported as well (Chopra et al., 2008). Potential harms of frequently or chronically high levels of the stress hormone cortisol include hippocampal atrophy, atherosclerosis (McEwen, 2007), and an unbalanced serotonergic and noradrenergic circuitry, resulting in serotonin depletion (Fig. 1) (Kopschina

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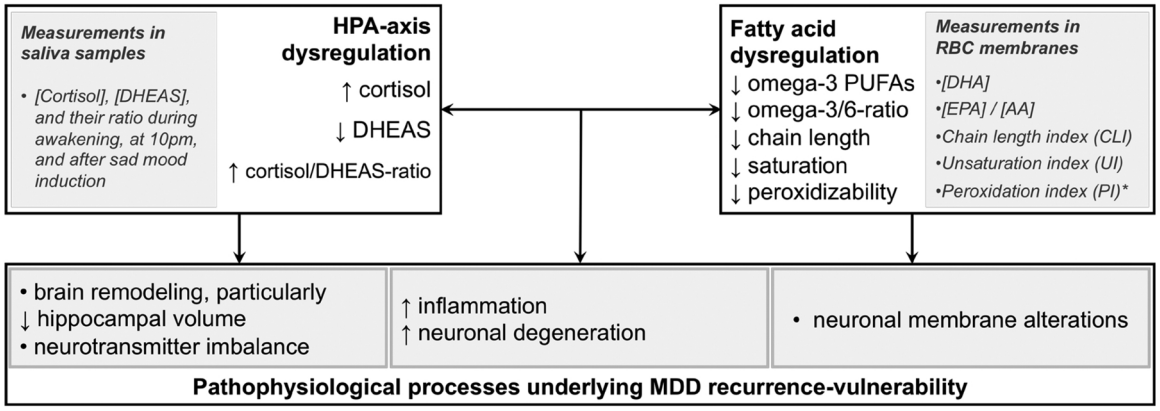

Figure 1. Schematic overview of hypothesized trait factors underlying MDD recurrence-vulnerability and their relations. This working hypothesis proposes that dysregulation of the principal stress system (HPA-axis) and dysregulation of overall fatty acid metabolism have a bidirectional relationship. Alterations in both systems could drive pathophysiological processes associated with MDD recurrence-vulnerability, including inflammation and neuronal degeneration. The measurements that were used to investigate HPA-axis activity and fatty acid metabolism in this study are indicated. *Abbreviations*: AA, arachidonic acid; DHA, docosahexaenoic acid; DHEAS, dehydroepiandrosterone sulfate; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids. *See Supplementary material S1 for the formulas used to calculate the fatty acid indices (CLI, UI, PI) (Kopschina Feltes et al., 2017; Maninger et al., 2009; McEwen, 2007; Mozaffarian and Wu, 2011; Piomelli et al., 2007).

Feltes et al., 2017).

Besides cortisol, adrenal glands secrete the neuroactive steroids dehydroepiandrosterone (DHEA) and its sulfate ester DHEAS, combinedly called DHEA(S). While its precise role remains unknown, DHEA(S) is thought to protect against the deleterious effects of elevated cortisol (Maninger et al., 2009). More specifically, while cortisol has catabolic properties, DHEA(S) is believed to have anabolic, neuroprotective, and regenerative effects on the brain (Maninger et al., 2009). Therefore, the cortisol/DHEA(S) ratio has been proposed to indicate HPA-axis balance (Chen et al., 2015). Interestingly, a low DHEAS and high cortisol/DHEAS ratio have been associated with MDD (Mocking et al., 2015a; Zhu et al., 2015), but conflicting results have been reported as well (Hough et al., 2017; Uh et al., 2017). Overall, increasing evidence suggests that HPA-axis alterations may be a trait factor in MDD patients that precede disease-onset (Goodyer et al., 2000), remains during remission, and can possibly predict recurrence (Lok et al., 2012; Mocking et al., 2015a).

Regarding the metabolic mechanisms that might underlie the pathophysiology of MDD, evidence suggests that alterations in polyunsaturated fatty acids (PUFAs) are important, specifically lower omega-3/omega-6 ratios (Lin et al., 2010; McNamara, 2009; Messamore and McNamara, 2016). Moreover, we previously showed broader alterations in the fatty acid profiles of recurrent MDD patients, including shorter, less saturated, and less peroxidizable fatty acids. These alterations did not only involve PUFAs, but also monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) (Assies et al., 2010; Mocking et al., 2012a, 2012b). Mechanistically, evidence exists for an essential role of fatty acids in cellular membrane processes, inflammation, and neural signaling (Fig. 1) (Mozaffarian and Wu, 2011; Piomelli et al., 2007). The brain is highly enriched in omega-3 DHA which is considered neuroprotective, due to its, amongst others, stimulation of brain-derived neurotrophic factor (Assies et al., 2014). Omega-3 EPA is the main precursor of anti-inflammatory eicosanoids, while omega-6 AA is considered the main precursor of pro-inflammatory eicosanoids. An imbalance between EPA and AA may lead to inflammatory dysregulation which is thought to be involved in the pathophysiology of MDD (Calder, 2006).

Interestingly, a bidirectional relation between fatty acids and HPA-axis alterations in MDD patients could be expected based on animal and preclinical studies (Chen and Su, 2013; Gounarides et al., 2008; Jazayeri et al., 2010; Delarue et al., 2003). Indeed, our previous cross-sectional research in recurrent MDD patients has shown a relationship between fatty acids and HPA-axis activity (Mocking et al., 2013). More specifically, evening cortisol concentrations were negatively associated with fatty acid unsaturation, chain length, and peroxidizability and these correlations were more negative in recurrent MDD patients compared with never-depressed controls. Our longitudinal research found a more negative relationship of fatty acid unsaturation and peroxidation with awakening cortisol in MDD patients compared with controls and these negative relationships were associated with antidepressant paroxetine nonresponse (Mocking et al., 2015b).

Based on this literature, HPA-axis activity, fatty acid metabolism, and their relationship might play important roles in the pathophysiology of (recurrent) MDD. Given the remaining inconsistencies and limited research on alterations in cortisol, DHEAS, and fatty acid metabolism in MDD during remission, we compared remitted recurrent MDD patients with matched never-depressed controls. We included only psychoactive medication-free subjects to exclude confounding effects of medication. The purpose of this study was to investigate: (I) whether cortisol, DHEAS, and fatty acid metabolism differ in remitted recurrent MDD patients compared to never-depressed controls (pointing to a potential trait factor) and (II) whether the association between cortisol, DHEAS, and fatty acid metabolism differed in remitted recurrent MDD patients as opposed to never-depressed control subjects.

2. Methods

2.1. Participants

We detailed the recruitment of the study population in our protocol paper (Mocking et al., 2016). In short, we recruited patients and controls from identical media announcements and from previous studies in several research centers across the Netherlands, including the Depression Evaluation Longitudinal Therapy Assessment (DELTA) study (Bockting et al., 2005). Inclusion criteria for patients were: ≥2 previous MDD episodes, as assessed by the Structured Clinical Interview for DSM-IV (SCID) (First, 1996); currently in remission for ≥8 weeks, defined by both a 17-item Hamilton Depression Rating Scale (HDRS) (Hamilton, 1960) ≤7 and not fulfilling the criteria for a current MDD episode as assessed by the SCID at inclusion; and age between 35 and 65 years. Exclusion criteria were: diagnoses of alcohol/drug dependency; any bipolar, psychotic, predominant anxiety, or severe personality disorder as assessed by the SCID; history of neurological disease or severe capital trauma; severe general illness; and the use of psychoactive drugs/medication < 4 weeks before assessments. The

control group included never-depressed participants without personal or first-degree familial psychiatric history as assessed by the SCID. Controls were carefully matched with the patient group for sex, age, educational level, employment status, and ethnicity. The accredited Medical Ethical Committee (METC) of the Academic Medical Centre (AMC) approved the protocol of this study. Prior to assessments, we obtained informed consent from all participants.

2.2. Measures

We measured depressive symptoms using the 17-item HDRS. We asked participants about age, gender, cohabitation situation, educational level, employment status, ethnicity, medication use (including contraceptives and dietary supplements) and smoking behavior and measured waist circumference, allowing us to correct for potential confounders.

2.2.1. HPA-axis

We instructed participants to provide five saliva samples during one day (at awakening, 30, 45, and 60 min thereafter, and at 22.00 h; Fig. S1) using Salivettes (Sarstedt AG and Co, Nümbrecht, Germany) to provide a stress-free reflection of blood cortisol and DHEAS concentrations (Kirschbaum and Hellhammer, 1994; Whetzel and Klein, 2010). Additionally, we collected saliva before and after sad mood provocation by combining a personal sad memory with sad music, as described previously (Mocking et al., 2016; Segal et al., 2006). In order to prevent saliva contamination by blood, we asked participants to refrain from brushing teeth or eating before saliva collection.

We determined cortisol and DHEAS concentrations by radioimmunoassay analysis designed for saliva samples (IBL, Hamburg, Germany; intra- and interassay variations: < 5 and $< 7\%$ for cortisol and < 10 and $< 12\%$ for DHEAS, respectively) and expressed concentrations in nmol/ml and ng/ml, respectively. We constructed cortisol and DHEAS awakening response curves and used the concentrations of cortisol and DHEAS to calculate their ratio.

2.2.2. Fatty acids

We drew blood during the lab visit after an overnight fast. We used erythrocyte fatty acid concentrations as a proxy for the fatty acid composition of neuronal membranes (Assies et al., 2010), a method previously described in more detail (Dacremont et al., 1995). Briefly, we separated, washed, and froze erythrocytes. Using capillary gas chromatography, we analyzed erythrocyte fatty acid concentrations expressed in pmol/10^6 erythrocytes. From the concentrations of 25 measured individual fatty acids, we calculated three structural indices in order to delineate main fatty acid patterns and to prevent type 1 errors from multiple testing (Mocking et al., 2012b), namely: (I) the chain length index (CLI) (mean number of carbon atoms per fatty acid); (II) the unsaturation index (UI) (mean number of double bounds per fatty acid; and (III) the peroxidation index (PI) (mean fatty acid susceptibility to oxidative stress) (see Supplementary material S1: fatty acid index formulas). In order to further prevent multiple testing, we a priori selected omega-3 docosahexaenoic acid (DHA), omega-3 eicosapentaenoic acid (EPA), and omega-6 arachidonic acid (AA) to delineate overall omega-3 and omega-6 fatty acid profiles in patients and controls as these are the main bioactive PUFAs in the brain, with both shared and independent effects (Calder, 2006). We provided the concentration of DHA and calculated the EPA/AA ratio in pmol/10^6 erythrocytes.

2.3. Data preparation

2.3.1. HPA-axis

Of the 73 patients, 9.6, 12.3, 12.3, 11.0, 8.2, 13.7, and 16.4% of the cortisol measurements and 12.3, 15.1, 12.3, 11.0, 8.2, 13.7, and 16.4% of the DHEAS measurements were missing at awakening, 30, 45, and 60 min after awakening, at 22 h, and before and after sad mood induction, respectively. Of the 46 controls, 8.7, 6.5, 6.5, 6.5, 6.5, 10.9, and 6.5% of the cortisol measurements and 10.9, 6.5, 6.5, 6.5, 6.5, 10.9, and 6.5% of the DHEAS measurements were missing, respectively. These percentages were lower compared to earlier data (range: 13.8–29.5%) (Mocking et al., 2013) and did not significantly differ between patients and controls ($p > 0.30$). All cortisol and DHEAS measurements approximated a normal distribution after log transformation, which we therefore used in all subsequent analyses. We considered cortisol or DHEAS concentrations that exceeded four standard deviations from the mean as missing, because this suggests saliva contamination with blood (Lok et al., 2012). No values had to be assigned as missing. We replaced three values with a z score of > 3.29 but < 4 SD by the highest observed nonoutlier value (Vijendra and Shivani, 2014).

2.3.2. Fatty acids

In our study, from all values of all 25 individual fatty acids, 4.7 and 5.2% were non-detectable in the patient and the control group, respectively. Similar to the HPA-axis measurements, these percentages were lower compared to earlier data (range: 13.8–29.5%) (Mocking et al., 2013). Nevertheless, in order to prevent bias introduced by missing data and to enable calculation of fatty acid structural indices for all participants—despite possible non-detects for individual fatty acids—we substituted non-detectable values by the minimum value observed for that particular fatty acid within our sample (Mocking et al., 2012b). For both groups, the highest amount of non-detects occurred in MUFAs and fatty acids with a relatively short carbon chain (C14–17), in agreement with earlier data (Mocking et al., 2012b).

We log-transformed the EPA/AA ratio to obtain normally distributed data. The fatty acid structural indices and DHA concentrations approximated normal distribution. Furthermore, we had to replace one value with a z score of > 3.29 with the highest observed non-outlier value.

2.4. Statistical analyses

We used IBM SPSS Statistics version 25 for our statistical analyses. We considered a p-value < 0.05 statistically significant. To compare sample characteristics between the patient and control groups, we used independent samples t-tests for continuous variables and χ^2-tests for categorical variables. In subsequent analyses, we controlled for confounders that were significantly associated with both group and the dependent (fatty acid or HPA-axis) variable, using linear mixed models. If a dichotomous (yes/no) confounder was present in $< 10\%$ of subjects, we excluded subjects with this confounder from the analyses. For example, omega-3 (fish oil) supplementation was found to be a confounder for awakening cortisol. As 11 out of 112 subjects (9.8%) indicated, they used omega-3 supplementation, we excluded these 11 subjects from our analyses on awakening cortisol in order to prevent confounding. All reported test statistics are corrected for confounders, unless otherwise specified.

To study potential MDD trait factors, we investigated differences between patients and controls in (I) cortisol, DHEAS, and their ratio (during the awakening response, in the evening and before and after sad mood induction), (II) fatty acid parameters (EPA/AA ratio, DHA concentrations, and fatty acid structural indices), and (III) their association.

First, to investigate differences in fatty acid parameters between patients and controls, we used independent samples t-tests, or linear mixed models in case we needed to correct for confounders. To investigate bimodal distributions, we inspected histograms. In case histograms suggested bimodal distributions, we proceeded with formal testing as described earlier (Mocking et al., 2012a).

Second, to assess whether MDD is associated with HPA-axis alterations, we used linear mixed models, with cortisol, DHEAS, or their ratio as dependent variable and sampling moment (awakening, 30, 45, and

60 min after awakening; in a separate model before or after sad mood induction), group (patient/control), and the sampling moment × group interaction term as independent variables. We used mixed models to incorporate correlations between repeated measurements in the same participant, thereby limiting occurrence of type I errors from multiple testing. A significant sampling moment × group interaction term would indicate that the group (patient/control) variable is associated with the course of cortisol or DHEAS over time. In case the sampling moment × group interaction term was not significant, we removed this term and tested the more parsimonious model. For HPA-axis evening activity, we investigated differences in cortisol and DHEAS concentrations, and their ratio at 22 h using independent samples *t*-tests. In post hoc analyses, we investigated differences between patients and controls using independent samples *t*-tests regarding (I) awakening cortisol concentrations and (II) the absolute increase in cortisol after awakening, calculated by subtracting the awakening from the 30 min after awakening values.

Third, to study the association between HPA-axis and fatty acid characteristics, we used multiple separate linear mixed models with sampling moment, group, the fatty acid parameter (EPA/AA ratio, DHA concentration, CLI, UI, or PI, respectively), the sampling moment × group interaction term, the sampling moment × fatty acid parameter interaction term, the group × fatty acid parameter interaction term, and the sampling moment × group × fatty acid parameter interaction term as independent variables and the HPA-axis characteristic (cortisol, DHEAS, or their ratio) as dependent variable. In case an interaction term was not significant, we removed this term and tested the remaining more parsimonious model. For graphical representation, we dichotomized (median split high versus low) the independent fatty acid variable in order to plot the relation between independent (fatty acid) variable and the dependent (HPA-axis) variable over the sampling moments in one figure. While all significant test statistics were reported, we only showed graphical representations of those associations that remained significant after dichotomization.

Finally, we performed post hoc regression analyses within the patient group with duration of current remission as independent variable and different HPA-axis and fatty acid characteristics as dependent variables.

3. Results

3.1. Characteristics of participants

Seventy-three patients and 46 controls initially participated; blood was collected and analyzed from 68 patients and 44 controls, and saliva from 67 patients and 43 controls, respectively. Table 1 shows population characteristics of patients and controls. The median number of previous major depressive episodes was 4 (range = 2–60, interquartile range = 5). Matching was successful for sex, age, educational level, employment status, and ethnicity. Additionally, no significant differences were observed between the MDD patients and controls in IQ, cohabitation situation, smoking behavior, and waist circumference. However, patients significantly differed from controls on three characteristics: higher residual depressive symptoms (HDRS score), higher use of omega-3 PUFA (fish oil) supplementation, and lower degree of current employment. During the analyses of each dependent variable, these three characteristics were tested as confounders and test statistics were corrected if needed.

3.2. HPA-axis activity in patients versus controls

Compared to controls, remitted recurrent MDD patients showed a higher cortisol awakening response ($p = 0.006$; Fig. 2A). Post hoc analyses showed that cortisol concentrations were significantly lower at awakening and increased significantly steeper between awakening and 30 min thereafter in patients compared to controls ($t_{106} = 2.155$, $p = 0.033$ and $t_{103} = -0.2754$, $p = 0.007$, respectively; independent samples *t*-tests). The DHEAS awakening curve did not differ in patients compared to controls ($p > 0.332$; Fig. 2B). Furthermore, the cortisol/DHEAS ratio awakening response followed a different course in patients versus controls ($p = 0.036$; Fig. 2C) with a steeper increase in the first 30 min after awakening and a steeper decrease afterwards.

Evening cortisol did not differ between patients and controls ($p = 0.348$), evening DHEAS was higher in patients at trend level ($p = 0.051$), and evening cortisol/DHEAS ratio was significantly lower in patients relative to controls ($p = 0.044$) (Fig. 2). HPA-axis responsiveness to sad mood induction did not differ between patients and controls for cortisol and DHEAS concentrations, or their ratio (p's > 0.244). Of note, cortisol significantly decreased after sad mood induction in both groups (sampling moment main effect: $F_{1,89.006} = 12.148$, $p = 0.001$). Correction for potential confounders did not yield different results.

Post hoc regression analyses within the patient group showed no significant associations between duration of current remission and HPA-axis characteristics.

3.3. Fatty acids in patients versus controls

Patients did not differ from controls in EPA/AA ratio, DHA concentrations, and fatty acid structural indices ($p > 0.395$). HDRS score was identified as a potential confounder for the CLI, and omega-3 PUFA (fish oil) supplementation for the PI, the EPA/AA ratio, and DHA concentrations. Separate sensitivity and mixed model analyses showed no different results when taking these confounders into account (Table 2). In contrast to earlier findings (Mocking et al., 2012a), no bimodal distributions for any of the fatty acid parameters were observed. Post hoc regression analyses within the patient group showed no significant associations between duration of current remission and fatty acid characteristics.

3.4. Association between HPA-axis and fatty acids

Differences in the association between HPA-axis activity and fatty acids between patients and controls are reported below. Findings on associations in the overall sample (in both patients and controls) are shown in the Supplementary Table S1.

Associations between awakening cortisol and fatty acid parameters did not differ between patients and controls ($p > 0.35$).

For awakening DHEAS (as dependent variable), however, the interaction terms of group × EPA/AA ratio, group × DHA, group × UI, and group × PI were significant (Fig. 3; Table 3). Associations between overall awakening DHEAS concentrations and these fatty acid parameters were significantly negative in patients, and positive in controls. This indicates that higher overall awakening DHEAS concentrations were associated with lower levels of fatty acid parameters in patients, while being associated with higher levels of these fatty acid parameters in controls.

Similarly, the awakening cortisol/DHEAS ratio (as dependent variable) was significantly associated with the interaction terms of group × EPA/AA ratio, group × DHA, group × UI, and group × PI (Table 3). These associations were oppositely directed in patients versus controls as well. Associations between the awakening cortisol/DHEAS ratio and these fatty acid parameters were significantly positive in patients, whereas negative in controls. This means that a higher awakening ratio of cortisol/DHEAS was associated with increased levels of these fatty acid parameters in patients, but with decreased levels in controls. Awakening DHEAS and cortisol/DHEAS ratio were not associated with the group × CLI interaction term ($p \geq 0.275$). Furthermore, evening cortisol was not associated with any group × fatty acid parameter interaction term ($p > 0.240$). Evening DHEAS, however, was significantly associated with the group × PI interaction term (Table 3; Fig. 4). The association between evening DHEAS and PI was

Table 1
Sample characteristics.

Characteristics		MDD-R (n = 68)	NC (n = 44)	Between-group statistic			
				χ^2	t	U	p
Female	N (%)	45 (66.2%)	30 (68.2%)	0.05			0.83
Age	Years; mean (SD)	53.5 (7.7)	51.5 (8.2)		−1.30		0.2
Education	Levels[a]	0/0/0/4/22/26/16	0/0/0/1/16/18/8	1.30			0.73
IQ	Mean (SD)	109 (7.8)	106 (9.6)			927	0.06
Cohabitation situation	Levels[b]	28/0/19/17/2/0/0	12/0/16/11/4/0/0	3.93			0.27
Employment status	Levels[c]	25/27/16/0	21/17/5/0	2.9			0.24
Currently employed	N (%)	45 (68.2%)	37 (86.0%)	4.46			**0.04**
Currently smoking	N (%)	13 (19.4%)	8 (18.6%)	0.01			0.92
Waist circumference	cm; mean (SD)	95.0 (13.9)	90.6 (15.1)		−1.53		0.13
Dutch ethnicity	N (%)	48 (75.0%)	35 (81.4%)	0.61			0.44
West-European ethnicity	N (%)	57 (83.8%)	36 (83.7%)	0.00			0.99
Omega-3 suppl.	N (%)	10 (14.9%)	1 (2.3%)	4.62			**0.03**
Statins use	N (%)	10 (14.9%)	3 (7.0%)	1.59			0.21
Vitamin D suppl.	N (%)	20 (29.9%)	10 (23.3%)	0.57			0.45
Oral anticonceptive use	N (%)	4 (5.9%)	5 (11.6%)	1.17			0.3
Age of onset	Years; mean (SD)	26.8 (10.9)	NA				
HDRS	Mean (SD)	2.69 (2.4)	1.02 (1.39)		−4.19		< **0.001**
Episodes	Median (IQR) min.–max.	4.0 (2/4/7) 2–60	NA				
Duration of remission	Years; Median (IQR) min. - max.	3.0 (1/3/7) 0–21	NA				

Abbreviations: NC, never-depressed control; HDRS, Hamilton Depression Rating Scale; IQR, inter-quartile range; MDD-R, recurrent major depressive disorder; p, p-value; T, independent-samples t-test statistic; U, Mann–Whitney U non-parametric test statistic; χ^2, Chi-square test statistic.
Bold values correspond to a P-value < .05.
[a] Level of educational attainment (Verhage, 1964): levels range from 1 to 7 (1 = primary school not finished; 7 = pre-university/university degree).
[b] Cohabitation situation: alone/living with parents/cohabiting/cohabiting with children/single living with children/other/unknown.
[c] Employment status: low/middle/high/never worked.

significantly negative in patients while positive in controls, meaning that a higher DHEAS was associated with a lower PI in patients, but with a higher PI in controls. Evening DHEAS was also marginally associated with the group × EPA/AA ratio interaction term (p = 0.056; Table 3). Finally, patients and controls did not differ in their associations between the evening cortisol/DHEAS ratio and fatty acids (p > 0.063).

4. Discussion

The current study investigated differences between 68 remitted unmedicated recurrent MDD patients and 44 never-depressed controls in cortisol, DHEAS, fatty acid metabolism, and their relation. Regarding HPA-axis activity, patients exhibited lower baseline salivary cortisol, but higher cortisol and cortisol/DHEAS ratio awakening responses compared to controls. Additionally, patients displayed a lower evening cortisol/DHEAS ratio. Patients did not differ from controls in erythrocyte fatty acid parameters. Nevertheless, the relation between HPA-axis and fatty acids differed between patients and controls, with a negative association between awakening DHEAS and EPA/AA ratio, DHA concentrations, UI, and PI in patients, while these relations were positive in controls.

4.1. HPA-axis activity in patients versus controls

Our findings confirm that a steeper cortisol awakening response may be a potential trait factor in remitted MDD patients that could indicate an increased biological vulnerability for (recurrent)

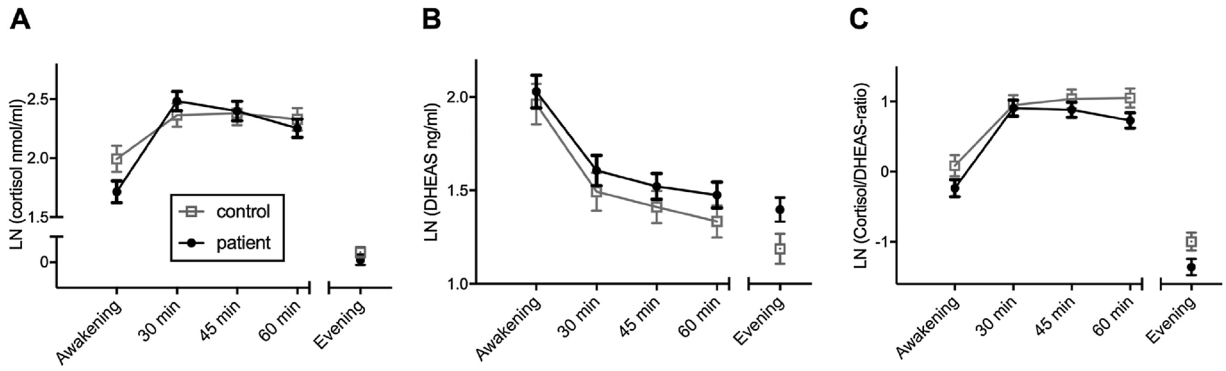

Figure 2. Cortisol and DHEAS concentrations and cortisol/DHEAS ratio during the awakening response and in the evening for remitted MDD-R patients compared to controls. Cortisol awakening results are adjusted for current employment (yes/no) and with subjects taking oral fish oil supplementation (n = 11) excluded. Mixed model analyses awakening results: for natural log transformed cortisol: sampling moment × group (patient/control) interaction term $F_{3,91.037}$ = 4.362, p = 0.006; for natural log transformed DHEAS: group main effect: $F_{1,107.199}$ = 0.950, p = 0.332; sampling moment main effect: $F_{3,100.313}$ = 43.840, p < 0.001; group × sampling moment interaction term: $F_{3,100.313}$ = 0.177, p = 0.912; for natural log transformed cortisol/DHEAS ratio: sampling moment × group interaction term $F_{3,102.174}$ = 2.959, p = 0.036. Independent samples t-tests evening results: for natural log transformed cortisol: t(108) = 0.943, p = 0.348; for natural log transformed DHEAS: t(108) = −1.965, p = 0.051; for natural log transformed cortisol/DHEAS-ratio: t(108) = 2.039, p = 0.044.

Table 2
Fatty acid parameters compared between MDD-R patients and controls.

	MDD-R (n = 68)		NC (n = 44)		Between-group statistics			
	Mean	SD	Mean	SD	t	F	df	p
CLI[a]	18.739	0.010	18.743	0.013		0.064	1,107[b]	0.802
UI	1.436	0.0480	1.435	0.0507	−0.193		110	0.847
PI[c]	1.239	0.0783	1.239	0.0746	0.017		99	0.986
EPA/AA ratio[c,d]	−3.187	0.4902	−3.128	0.5062	0.586		99	0.559
DHA[c]	23.138	6.5105	22.565	6.6179	−0.434		99	0.665

Abbreviations: AA, arachidonic acid; C20, 4 omega-6; CLI, chain length index; df, degrees of freedom; DHA, docosahexaenoic acid; C20, 6 omega-3; EPA, eicosapentaenoic acid; C20, 5 omega-3; F, fixed effect of group (patients/controls); NC, never-depressed controls; MDD-R, remitted, recurrently depressed patients; p, p-value; PI, peroxidation index; T, independent-samples t-test statistic; SD, standard deviation; UI, unsaturation index.

[a] CLI results are adjusted for natural log transformed HDRS score using linear mixed models.

[b] Numerator df and denominator df.

[c] Subjects taking omega-3 PUFA (fish oil) supplementation (N = 11) are excluded for the PI, EPA/AA ratio and DHA results.

[d] Natural log transformation.

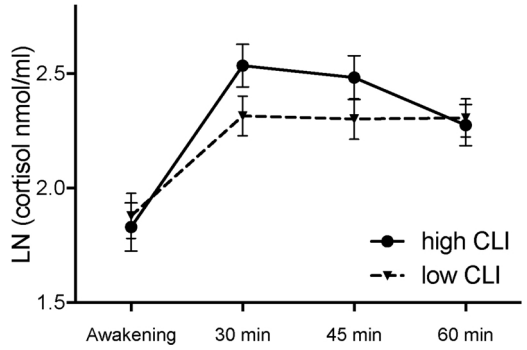

Figure 3. Cortisol concentrations during the awakening response in subjects (patients and controls combined) with high versus low fatty acid carbon chain lengths. Mixed model analysis results: sampling moment × N_CLI interaction term: $F_{3,90.112} = 5.815$, $p = 0.001$. *Abbreviations:* N_CLI, median split (high versus low) dichotomized variable of fatty acid carbon chain length. Results are adjusted for current employment (yes/no) and with subjects taking oral fish oil supplementation (n = 11) excluded.

Table 3
Linear mixed model analyses statistics for the significant associations between HPA-axis and fatty acids in patients versus controls.

Dependent variable	Independent variable	Mixed model statistics	
		F	p
Awakening DHEAS	Group × EPA/AA ratio	9.340	0.003
	Group × DHA	8.650	0.004
	Group × UI	9.249	0.003
	Group × PI	12.169	0.001
Awakening cortisol/DHEAS ratio	Group × EPA/AA ratio	6.282	0.014
	Group × DHA	4.600	0.034
	Group × UI	5.770	0.018
	Group × PI	8.374	0.005
Evening DHEAS	Group × EPA/AA ratio	3.741	*0.056*
	Group × PI	5.051	0.027

Abbreviations: AA, arachidonic acid; C20, 4 omega-6; CLI, chain length index; DHA, docosahexaenoic acid; C20, 6 omega-3; DHEAS, dehydroepiandrosterone sulfate; EPA, eicosapentaenoic acid; C20, 5 omega-3; F, fixed effect of group (patients/controls); p, p-value; PI, peroxidation index; UI, unsaturation index.

depression. In line with our findings, Vreeburg et al. (2009) demonstrated higher cortisol awakening responses and normal evening cortisol concentrations in a large sample of remitted MDD-patients. Our study corroborates this finding in a specific subgroup of highly recurrent remitted MDD patients while excluding the confounding effect of psychoactive medication (Parrott and Downey, 2017). However, while Vreeburg et al. (2009) showed normal baseline cortisol concentrations, our results demonstrate a lower morning baseline cortisol in patients, in accordance with research in another population of remitted recurrent MDD patients (Ahrens et al., 2008). To complicate matters, Lok et al. (2012) observed hypercortisolaemia in remitted recurrent MDD, but did not exclude potential confounding by psychoactive medication.

These discrepancies might partially result from conceptual heterogeneity of patients as many different combinations of symptoms could lead to the DSM diagnosis of MDD (Olbert et al., 2014). However, conflicting findings may also be explained by methodological factors (sampling time/frequency/laboratory measures) and quality of patient–control matching. In comparison to earlier studies (Ahrens et al., 2008; Lok et al., 2012; Vreeburg et al., 2009), the current study included matched groups for smoking habits, waist circumference, and demographic factors such as age, sex, ethnicity, and educational level and consisted of seven salivary assessments to provide a reflection of diurnal HPA-axis patterns, thereby reducing effects of potential confounders and methodological factors. In sum, although our study adds new evidence (a steeper cortisol awakening response in MDD), inconsistencies in research performed thus far regarding HPA-axis alterations in MDD preclude firm conclusions.

4.2. Fatty acids in patients versus controls

Contrary to our expectations, results did not show alterations in EPA/AA ratio, DHA concentrations, or fatty acid structural indices in patients compared to controls. Findings regarding fatty acids in MDD thus far have been inconsistent, with previous studies reporting both lower fatty acid indices in remitted recurrent patients (Mocking et al., 2012a, 2012b) and higher fatty acid indices in acute patients (Mocking et al., 2015b). Similarly, while general findings in the literature report decreased EPA and DHA concentrations in acute MDD (Messamore and McNamara, 2016), comparable concentrations in patients and controls have been reported as well (Mocking et al., 2015b; Lin et al., 2010). Previous, more heterogeneous samples might have overestimated fatty acid abnormalities, as patients better matched controls in the current study on, for example, waist circumference. Interestingly, compared to results of Mocking et al. (2012a, 2012b), the fatty acid structural indices and omega-6 AA concentrations are higher in the overall sample (patients/controls) of the present study despite using identical methodology. This may possibly be related to a higher waist circumference in both groups in our current sample. This could reflect a further change in modern Western diets, which have shown a steep increase in omega-6 fatty acid intake over the last decades. Higher intakes of omega-6 PUFAs have been associated with obesity and would indeed cause higher fatty acid structural indices (Simopoulos, 2016). This notion is supported by higher concentrations of omega-6 fatty acids and a

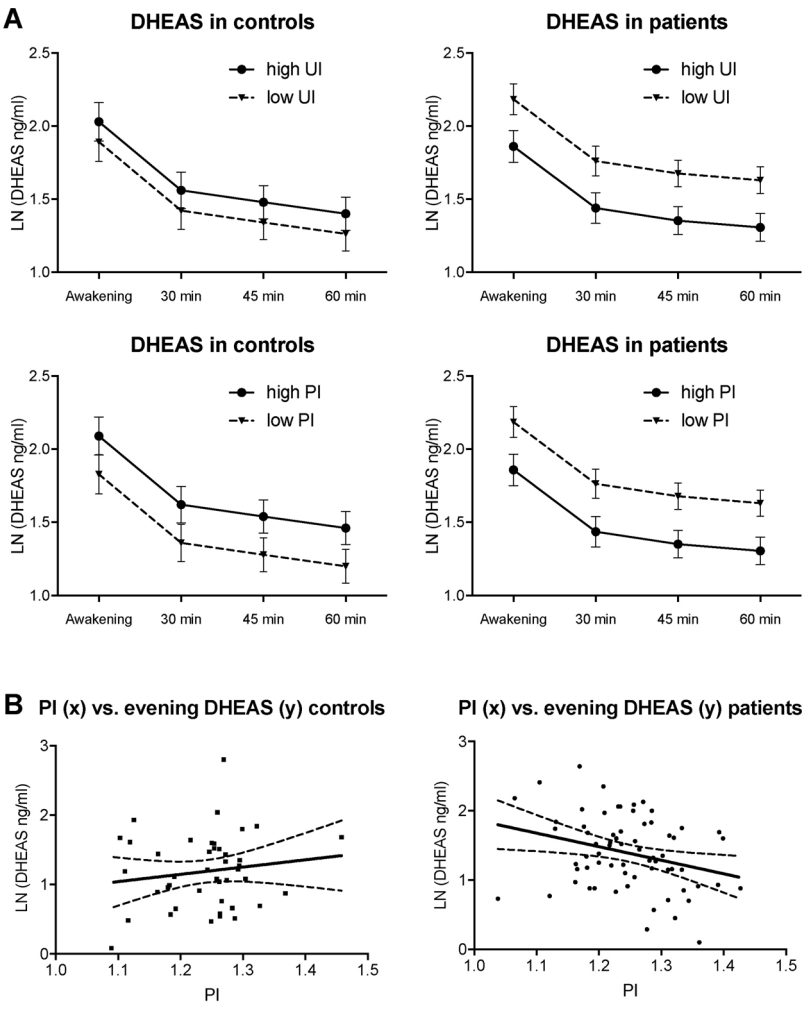

Figure 4. (A) DHEAS concentrations during the awakening response in patients and controls with a high versus low unsaturation index (UI) and a high versus low peroxidation index (PI). Mixed model analysis results: group × N_UI interaction term: $F_{1,105.218} = 5.043$, $p = 0.027$; group × N_PI interaction term: $F_{1,105.180} = 8.449$, $p = 0.004$. *Abbreviations:* N_UI, median split (high versus low) dichotomized variable of fatty acid unsaturation; N_PI, median split (high versus low) dichotomized variable of fatty acid peroxidizability. (B) The association between evening DHEAS concentrations and fatty acid peroxidizability in patients compared to controls. Mixed model analysis results: group × PI interaction term: $F_{1,106.000} = 5.051$, $p = 0.027$.

positive relation between waist circumference and the CLI ($p = 0.014$) in the current sample.

4.3. Associations in HPA-axis activity and fatty acids in patients versus controls

Interestingly, we found that awakening DHEAS was negatively related with fatty acid parameters (EPA/AA ratio, DHA, UI, PI) in patients, while this relation was positive in controls. To the best of our knowledge, this is the first study in MDD patients investigating associations between DHEAS (or cortisol/DHEAS ratio) and fatty acid parameters. Based on preclinical studies in which DHEA(S) administration was associated with a decrease in omega-6/omega-3-ratios and fatty acid saturation in rat (de Heredia et al., 2009) and human (Hernandez-Morante et al., 2010) adipose tissue, we hypothesized that higher DHEAS concentrations would correlate with higher omega-3 DHA concentrations, a higher EPA/AA ratio, and higher fatty acid unsaturation. In line with this, Assies et al. (2003) showed that DHEA supplementation in X-linked adrenoleukodystrophy patients with impaired peroxisomal fatty acid beta-oxidation resulting in accumulation of very long chain fatty acids in the nervous system led to a decrease in erythrocyte omega-6 fatty acids. This positive relation was indeed found in our control group, representing the physiological situation.

One could interpret the reversal of this relation in the MDD patients by considering that (I) although we did not find differences in fatty acid metabolism in patients versus controls, patients might still suffer from subclinical/functional fatty acid dysregulation, and/or (II) DHEAS upregulation could be protective against fatty acid dysregulation (Assies et al., 2003). From this perspective, DHEAS upregulation could represent an adaptive response to subtle fatty acid aberrations; with more severe aberrations leading to higher DHEAS concentrations. If this is true, DHEAS upregulation appears to fail in completely correcting fatty acid dysregulation in patients, resulting in a negative relation rather than the positive association found in controls. However, since this is the first study to investigate this relation in MDD and our results on fatty acids are inconsistent with previous literature, these findings should be replicated first and interpreted thereafter.

4.4. Clinical and research implications

Current literature on cortisol and fatty acid metabolism in MDD reports discrepancies. Research on DHEA(S) in MDD, and especially its relation to fatty acids, remains sparse. Therefore, results of this study should be replicated to investigate the reproducibility of our findings. Specifically, prospectively repeated measurements would allow for the investigation of the persistence and predictive value of the alterations identified in the current study. Moreover, it would be interesting to also study the relation between DHEA(S) and fatty acids during a depressive episode. In such a design, the relation between severity of depressive symptoms and biological alterations could be tested using regression analyses and compared to the relation in controls. Particularly repeated measures in the same subjects during remission and during depressive episodes could disentangle state and trait effects. Additional ecological momentary assessments could shed light on the influence of subjective stress experiences on the HPA-axis (Shiffman et al., 2008).

Furthermore, the inconsistent picture that arises from our and

previous results does not provide evident biomarkers or intervention targets for clinical use and temper expectations of clinical trials targeting fatty acid profiles (e.g. lipid status as predictive biomarker; Parekh et al., 2017) or the HPA-axis (e.g. administration of cortisol biosynthesis inhibitors; McAllister-Williams et al., 2016).

Moreover, the reversed relation between DHEAS and fatty acids can be seen as evidence for alterations in underlying metabolic networks. Investigating these complexly interacting metabolic pathways may provide more powerful information, as it allows for delineating affected pathophysiological pathways from a multivariate perspective. Network analyses may identify nodes in the network that can be effectively targeted to restore homeostasis.

Finally, an adaptive upregulation of DHEAS has been proposed in MDD (Uh et al., 2017; Hough et al., 2017). This fits with our finding of a lower evening cortisol/DHEAS ratio in remitted recurrent MDD patients. Together, this could suggest that higher DHEAS concentrations, relative to cortisol, may be a trait factor indicating resilience rather than vulnerability. However, given the cross-sectional design, our findings preclude causal conclusions. It would be interesting to test the prospective predictive value for recurrence of DHEAS and cortisol/DHEAS ratio in MDD during remission.

4.5. Limitations and strengths

Some limitations of the current study need to be addressed. First, we did not include dietary data. Therefore, potential confounding effects of dietary effects may have been missed. However, patients did not differ from controls in DHA concentrations and EPA/AA ratio, suggesting that if a dietary confounding effect exists, this would have led to masking of differences rather than overestimating them. Moreover, diet has been associated with demographic and lifestyle factors such as educational level and waist circumference (Knudsen et al., 2014). Because patients and controls were matched for those factors, the potential confounding role of diet may have thereby been reduced as well.

Second, the exclusion of patients using psychotropic drugs may have led to the selection of particular patient subgroups, e.g. patients who previously experienced little benefit or side effects from antidepressant medication. However, we deemed this exclusion criteria necessary in order to investigate our research questions while eliminating the potential confounding effects of antidepressants. In addition, also the other inclusion and exclusion criteria, for both patients (e.g. age) and controls (no psychiatric familial history), may have limited the extrapolation of our findings. Nevertheless, we applied these criteria to include a relatively homogeneous sample of patients and compare them to a never-depressed control sample that is at relatively low risk of developing MDD.

Finally, our sad mood induction method (Mocking et al., 2016) did not succeed in activating the HPA-axis in patients nor controls. Unexpectedly, cortisol concentrations even significantly lowered after sad mood provocation. As a consequence, these data were not included in subsequent analyses to investigate the association between HPA-axis activity and fatty acids. These results contradict the general findings on psychological stressors in MDD, which have consistently reported an elicited cortisol response in both patients and controls (Burke et al., 2005; Lange et al., 2013). However, most studies applied public speaking or cognitive tasks as a stressor. Interestingly, Chopra et al. (2008) applied the same sad mood challenge as was used in the present study and found similar results of a decreased stress response in both patients and controls. Together, this could suggest that anticipation of the sad mood provocation might cause a higher stress response than the method itself. In addition, it may be that the induced sad mood was not perceived as stressful by the subjects, but rather as a pleasant melancholic state. Future studies should consider assessing (I) anticipatory stress by measuring whether HPA-axis activity increases prior to sad mood provocation and (II) how the mood-induction procedure was perceived by the subjects.

Strengths of our study are the inclusion of a specific highly vulnerable group of remitted patients with two or more previous MDD episodes (median four), thereby representing a sample with high recurrence risk without distortion due to confounding effects of residual MDD symptoms or psychoactive medication use. Furthermore, we are the first to investigate the relationship between fatty acid metabolism and DHEAS or cortisol/DHEAS ratio in MDD.

5. Conclusion

Compared to never-depressed matched controls, remitted recurrent MDD patients showed a lower baseline, but higher cortisol awakening response, especially relative to DHEAS concentrations. This corroborates with earlier findings of higher HPA-axis responsiveness in MDD and may represent a trait marker for MDD-recurrence-vulnerability that remains during remission. Moreover, awakening DHEAS concentrations were negatively associated with omega-3 fatty acid profiles and fatty acid unsaturation and peroxidizability in patients, while these associations were positive in controls.

This study adds evidence that cortisol, DHEAS, and their relation with fatty acid metabolism are altered during remission in a subgroup of highly recurrent MDD patients. Future prospective research is warranted to explain inconsistencies and lead to development of recurrence-vulnerability biomarkers.

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Author contributions

All authors were involved in conception of the study, interpretation of results, preparation, critical revision, and approval of the final manuscript. C.A.F. and R.J.T.M. were involved in acquisition of the data. D.M.H. and R.J.T.M. performed statistical analyses. D.M.H. and R.J.T.M. had access to all the data in the study and take full responsibility for both the integrity of the data and the accuracy of the data analysis.

Conflicts of interest

All authors report no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.psyneuen.2018.10.012.

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