Fatty acids in context

Neurometabolic perspectives on depression vulnerability

Mocking, R.J.T.

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Relationship between the hypothalamic-pituitary-adrenal-axis and fatty acid metabolism in recurrent depression.

ABSTRACT

Alterations in hypothalamic-pituitary-adrenal (HPA)-axis activity and fatty acid metabolism have been observed in (recurrent) major depressive disorder (MDD). Through the pathophysiological roles of fatty acids in the brain and cardiovascular system, a hypothesized relationship between HPA-axis activity and fatty acid metabolism could form a possible missing link accounting for the association of HPA-axis hyperactivity with recurrence and cardiovascular disease in MDD.

In 137 recurrent MDD-patients and 73 age- and sex-matched controls, we therefore investigated associations between salivary cortisol (morning and evening) and the following indicators of fatty acid metabolism measured in the red blood cell membrane: (I) three main fatty acids [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA)], and (II) structural fatty acid indices (unsaturation, chain length, peroxidation) calculated from concentrations of 29 fatty acids to delineate overall fatty acid characteristics. In addition, we compared these associations in patients with those in controls.

In patients, evening cortisol concentrations were significantly negatively associated with DHA ($\beta = -1.358; SE = 0.499; t = -2.72; P = .006$), the unsaturation index ($B = -0.021; SE = 0.009; t = -2.42; P = .018$), chain length index ($\beta = -0.060; SE = 0.025; t = -2.41; P = .019$), and peroxidation index ($\beta = -0.029; SE = 0.012; t = -2.48; P = .015$). The relations between cortisol and the latter three variables were significantly more negative in patients relative to controls. Significance remained after correction for confounders.

Our results suggest a relationship between HPA-axis activity and fatty acid metabolism in recurrent MDD. Future randomized experimental intervention studies using clinical outcome measures could help to further elucidate the suggested effects of hypercortisolemia in the brain and cardiovascular system in recurrent MDD.
INTRODUCTION

Major depressive disorder (MDD) accounts for an overwhelming global burden of disease. This is mainly due to its (I) lifelong recurrent nature, and (II) association with cardiovascular comorbidity. Yet, pathophysiological pathways underlying the recurrent nature of MDD and its association with cardiovascular disease remain unclear. A potential missing link could be a relationship between hypothalamic-pituitary-adrenal (HPA)-axis activity and fatty acid metabolism.

The HPA-axis is the principal endocrinological stress axis, with the glucocorticoid hormone cortisol as the primary end product of its activation. Although mixed results exist, the extensive literature on cortisol in MDD mainly shows that MDD-patients exhibit higher cortisol concentrations than healthy controls, both during MDD-episodes and in remission, suggesting an endophenotype. This hypercortisolemic trait has been proposed to contribute to the development of both MDD-episodes and cardiovascular disease in MDD. Specifically, hypercortisolemia predicts the development of a first MDD-episode in subjects at risk, as well as recurrent episodes in remitted MDD-patients, possibly through the effects of excess cortisol on the brain, particularly the hippocampus. However, mixed results have also been observed. In addition, hypercortisolemia is predictive of prospective death from cardiovascular disease in MDD-patients. Nevertheless, the precise (patho)physiological pathways underlying the origin of hypercortisolemia and these associations with recurrence and cardiovascular disease in MDD remain unclear.

As for the HPA-axis, disturbed fatty acid metabolism has been consistently reported in MDD, both in acutely depressed and remitted patients. Main findings are lower concentrations of omega-3 long chain polyunsaturated fatty acids (LCPUFA) [e.g. eicosapentaenoic acid (C20:5 omega-3; EPA) and docosahexaenoic acid (C20:6 omega-3; DHA)], and decreased overall fatty acid unsaturation, chain length and peroxidizability.

Fatty acids have important structural and functional (patho)physiological roles in both the nervous and cardiovascular system. Structurally, fatty acids are major components of (neuronal) membranes. Unsaturation and chain length of membrane fatty acids determine membrane fluidity, which on its turn influences functioning of membrane bound proteins, e.g. neurotransmitter receptors and cardiac ion channels. Moreover, membrane fatty acid peroxidizability determines membrane susceptibility to oxidative stress. Functionally, fatty acids [particularly EPA, DHA and arachidonic acid (C20:4 omega-6; AA)] are involved in inflammatory regulation, and maintenance of brain cytoarchitecture.

Previous studies have found a modulating effect of HPA-axis activity on fatty acid metabolism. Cortisol influences mobilization, lipolysis, oxidation, and synthesis of fatty acids. For example, cortisol inhibits Δ5- and Δ6-desaturase-activity, enzymes responsible for unsaturation of fatty acid chains. In addition, oxidative stress associated with hypercortisolemia could influence fatty acid concentrations. These influences seem to have differential effects on specific fatty acids in such a way that high
cortisol concentrations are associated with a decrease in omega-3 LCPUFA concentrations and fatty acid unsaturation, chain length and peroxidizability.

Vice versa, fatty acids also seem to affect the HPA-axis. Dietary supplementation of omega-3 LCPUFA (e.g. EPA) reduced cortisol concentrations in rats, healthy subjects, and MDD-patients. In addition, a maternal preweaning omega-3 PUFA deficient diet induces HPA-axis hyperactivity in rat offspring. Furthermore, in chronically stressed monkeys, the omega-6/omega-3 ratio was positively associated with cortisol response to acute stress. Supplementation of omega-3 LCPUFA increases concentrations of EPA and DHA (polyunsaturated fatty acids with a long chain length), and decreases concentrations of AA. These fatty acid alterations may alter the feedback of the HPA-axis in three ways: (I) fatty acids influence glucocorticoid receptor functioning, depending on their degree of unsaturation and chain length, (II) EPA and AA modulate p-glycoprotein function and thereby cortisol transport across the blood-brain barrier, and (III) the AA/EPA ratio regulates production of pro- or anti-inflammatory eicosanoids, which can influence HPA-axis activation [via corticotrophin releasing hormone (CRH) secretion] and feedback (through induction of glucocorticoid receptor resistance).

Based on this literature, a relationship between the HPA-axis and fatty acid metabolism can be expected. Through the effects of fatty acids in the brain and cardiovascular system, this relationship might play an important role in the reinforcement and explanation of recurrence and cardiovascular disease in recurrent MDD.

However, the association between cortisol and fatty acid metabolism has never been investigated in MDD, especially not in comparison with controls. Therefore, we aimed to study the supposed relationship between HPA-axis activity and fatty acid metabolism in MDD by testing the associations between cortisol and fatty acid concentrations in patients with recurrent MDD and matched controls. We hypothesized that cortisol would be negatively associated with (I) concentrations of omega-3 LCPUFAs (e.g. EPA and DHA), and (II) indicators of overall fatty acid metabolism (e.g. unsaturation, chain length and peroxidizability). In addition, we hypothesized that these associations would be more negative in patients with recurrent MDD than in controls.

**METHODS**

**Participants**

Recruitment of the study population has been described in more detail previously. In brief, we initially recruited patients at psychiatric centers and through media announcements for participation in a randomized controlled trial investigating the effect of eight weekly group sessions of cognitive therapy versus naturalistic care on recurrence in patients with recurrent MDD. Inclusion criteria of the original trial were: ≥2 previous MDD-episodes in the last 5 years, as defined by the Structured Clinical Interview for DSM-IV disorders (SCID); in remission >10 weeks and <2 years, as defined by a score <10 on the 17-item Hamilton Depression Rating Scale (HDRS17); and 18–65 years old. Exclusion criteria were: (a history of) bipolar spectrum disorder or any psychotic disorder, organic brain damage, alcohol and/or drug abuse and/or dependency, or predominant anxiety disorder, all assessed by the SCID.
For the present study, we invited patients at 2 years follow-up of the original trial. Thereby, we aimed to recruit a homogeneous sample of patients with an endogenous biological vulnerability for recurrent MDD and cardiovascular comorbidity. We did not exclude patients based on depressive status or medication (e.g. antidepressants) use, because previously, both fatty acid metabolism and HPA-axis activity were not substantially influenced by these factors in this population. In addition, we recruited controls through media-advertisements, matched for sex and age using strata based on gender and 5-year age groups. We excluded controls with a current or past (personal and/or family) history of psychiatric DSM-IV axis-I disorders, as assessed by the SCID.

The medical ethical committee of the Academic Medical Center of the University of Amsterdam approved the study protocol, and all participants provided written informed consent.

Measures
To correct for potential confounders, we asked subjects for marital status, educational level, social class, smoking behavior, and calculated their body mass index (BMI; weight/length^2). We operationalized smoking dichotomously (yes/no), and educational level in three classes: low (primary education or preparatory middle-level applied education), middle (higher general continued education or middle-level applied education) and high (preparatory scientific education, higher applied education or scientific education). Similarly, we distinguished three social classes, based on occupation: Class 1, e.g. cleaner; Class 2, e.g. nurse; Class 3, e.g. general manager, as described previously. We measured depressive symptoms in all subjects using the HDRS.

HPA-axis
For the HPA-axis, we measured concentrations of cortisol, expressed in nmol/L, using radioimmunoassays (IBL Hamburg; designed for saliva samples; intra- and interassay variations: 5.1% and 6.5%, respectively) on saliva collected in neutral cotton salivettes (Sarstedt AG and Co, Nümbrecht, Germany), which provides a stress free, minimally intrusive, and reliable reflection of blood cortisol concentrations. We instructed subjects to collect saliva at home at three sampling moments on two consecutive days (day one: 0800 h and 2200 h; day two: 0800 h), after rinsing their mouths with water and not having brushed their teeth. Subjects collected morning samples after an overnight fast, and kept all samples refrigerated until sending them back by mail to the clinic on day two, where we stored samples at -20°C until analysis. For analyses, we averaged the two subsequent morning (0800 h) cortisol values into one morning cortisol value. Both averaged morning and evening cortisol were normally distributed after log transformation.

Fatty acid metabolism
We used washed red blood cell fatty acid concentrations as a model of brain fatty acid concentrations. From blood, sampled in the non-fasting state, we separated and washed red blood cells and stored them at -80°C until analyses by capillary gas chromatography, as described previously. We expressed concentrations of 29 different fatty acids in pmol/10^6 red blood cells. To analyze the association between HPA-axis activity and overall fatty acid metabolism, we calculated three indices that delineate main structural fatty acid characteristics on the basis of all 29 fatty acid concentrations. The (I) unsaturation
index (UI) denotes the mean number of double bounds per fatty acid; (II) chain length index (CLI), provides information about the mean number of carbon atoms per fatty acid; and (III) peroxidation index (PI), delineates the mean fatty acid susceptibility to oxidative stress.

Statistical analyses
To (I) prevent bias possibly introduced by missing values, and (II) facilitate calculation of indices - despite non-detectable fatty acid in individual patients - and thereby remaining adequate power, we used multiple imputation, as described previously. Simulation research has shown that multiple imputation effectively reduces bias potentially introduced by missing values, not by estimating these missing values themselves, but by providing highly valid estimations of effects parameters in a model based on partly missing data. As opposed to most other imputation techniques, it does include the uncertainty in the estimation of these effect parameters using a random term. By doing so, the estimation of the effect parameters is penalized through correction (increase) of the standard errors for the variance between the multiple imputation datasets. Multiple imputation is also reported to perform very well in small samples (N = 50), even with large multiple regression models (as large as 18 predictors) and even when as much as 50% of data in the dependent variable is missing.

In our study, in brief, 28.1%, 29.5%, 29.0%, 14.3%, 13.8% and 14.3% of the two morning and evening cortisol, EPA, DHA and AA values, respectively, were missing or non-detectable. As reported previously, we used Amelia II (http://www.gking.harvard.edu/amelia/), available as a package for R (http://www.r-project.org) to impute missing values. In this process we incorporated variables that are known to, or were observed to, be associated with the variables which had missing values. Specifically, we included sex, age, marital status, educational level, social class, HDRS17 score, weight, length, waist and hip circumference, smoking, and salivary cortisol and dehydroepiandrosterone sulphate, folic acid, vitamin B6 and B12, homocysteine, and all other measured fatty acid concentrations in our imputation model. Although Amelia II performs relatively well with non-normally distributed variables, we transformed non-normally distributed and categorical/ordinal variables as indicated by the program. In addition, for non-detectable values, we assigned range priors in Amelia II that indicate that a non-detectable fatty acid concentration must lie between 0.001 and the detection limit of that fatty acid (with 99% confidence). We used standard diagnostics available in Amelia II to check the imputation results, and observed no potential problems, e.g. induced by deviations from normal distribution.

To compare patients’ and controls’ basic characteristics, we used independent-samples t-tests and \( \chi^2 \)-tests. We additionally implemented multiple regression models, with morning or evening cortisol as independent variable and the fatty acid or index as outcome variable. We corrected for confounders, selected a priori, by adding relevant variables (sex, age, marital status, educational level, social class, HDRS17-score, BMI and smoking) as predictors to the model. In addition, although fatty acid and cortisol concentrations were not substantially influenced by antidepressant use or depressive state in this population, effects of these variables have been reported. Therefore, we additionally included depressive state (yes/no) and antidepressant use (yes/no) as predictors in the patient models. Moreover, since particularly tricyclic antidepressants (TCAs) have been reported to influence the HPA-axis,
we conducted additional sensitivity analyses in which we excluded TCA users from the analyses. To investigate the differences in HPA-axis-fatty acid metabolism associations between patients and controls, we used another set of regression analyses. These regression models additionally included [next to the group variable and the appropriate HPA-axis variable (morning or evening)] a group × HPA-axis (morning and evening in separate models) interaction in the model as an independent variable, with the different fatty acid metabolism variables as outcome variables. Finally, to provide an impression of the effects of multiple imputation on the analyses, we additionally performed the analyses without using multiple imputation, represented in Tables S1-S3.

RESULTS

Participant characteristics
One hundred and thirty-seven patients and 73 controls were included in this study. Table 1 shows characteristics of patients and controls. Matching for age and sex was successful, although patients differed on some demographic variables (lower educational level and social class in the patient group). Morning and evening cortisol values corresponded with a hyperactive HPA-axis in the patients. In addition, patients had a disturbed fatty acid metabolism, reflected by both lower concentrations of EPA, DHA and AA and lower fatty acid indices.\textsuperscript{16,24,27}

Association between HPA-axis and fatty acids in patients
Table 2 shows results of linear regression models of associations between morning and evening cortisol and fatty acid concentrations and indices in patients. All indices and DHA (Figure 1) were negatively associated with evening cortisol concentrations (.006 ≤ \(P\) ≤ .019); significance remained after correction for possible confounders (.003 ≤ \(P\) ≤ .023), and after exclusion of TCA users. This indicates that more pronounced evening hypercortisolemia was associated with lower concentrations of the omega-3 LCPUFA DHA and decreased overall fatty acid unsaturation, chain length and peroxidizability. Morning cortisol concentrations were not significantly associated with fatty acid metabolism. Results without multiple imputation are provided in Table S1.

Association between HPA-axis and fatty acids in controls
Table 3 shows results of linear regression models of associations between morning and evening cortisol and fatty acid concentrations and indices in controls. Morning cortisol was positively associated with AA at trend level (\(P = .059\)), other associations between cortisol and fatty acid metabolism were not significant (.518 ≤ \(P\) ≤ .985; Table 3). Results without multiple imputation are provided in Table S2.

Differences between patients and controls in the association between HPA-axis and fatty acids
The group × evening cortisol interaction-term was significant for the UI, CLI and PI (.024 ≤ \(P\) ≤ .032; Table 4; Figure 2). Associations between evening cortisol concentrations and index measures were significantly negative in patients relative to controls. This indicates that higher evening cortisol concentrations were associated with greater decreases in overall fatty acid unsaturation, chain length and peroxidizability in patients relative to controls.
These differences remained after correction for confounders \(0.034 \leq P \leq 0.049\). Results remained significant after exclusion of TCA users, except for the corrected effect of the group × evening cortisol interaction regarding the PI \(P = 0.063\). The group × evening cortisol interaction-term was not significant for the individual fatty acid concentrations \((P > 0.127)\). The group × morning cortisol interaction was not significant for any of the indicators of fatty acid metabolism. Results without multiple imputation are provided in Table S3.

**DISCUSSION**

In this study, we investigate the relationship between HPA-axis activity and fatty acid metabolism in recurrent MDD by testing associations between cortisol and fatty acid concentrations in 137 patients with ≥2 previous MDD-episodes and comparing them with 73 matched controls. Consistent with our hypotheses, evening cortisol concentrations were significantly negatively associated with the omega-3 LCPUFA DHA, and overall fatty acid unsaturation, chain length and peroxidizable in the patients. Furthermore, we found that the associations of evening cortisol with fatty acid indices were significantly different in MDD-patients versus controls (group × HPA-axis interaction), with significantly negative associations in MDD-patients relative to controls.

The associations between cortisol and DHA, fatty acid unsaturation, chain length and peroxidizable underlie the relationship between functioning of the HPA-axis and fatty acid metabolism in MDD. The association between DHA and cortisol is in line with earlier clinical observations of associations between DHA and indicators of HPA-axis activity: DHA was associated with CRH in perpetrators of domestic violence, and \(5\alpha\)-dihydroprogesterone in patients diagnosed with either alcohol abuse, MDD, or both.

Studies that experimentally influenced this relationship between HPA-axis activity and fatty acid metabolism by administration of fatty acids or glucocorticoids suggest that effects could operate in two directions: on the one hand, an effect of the HPA-axis on fatty acid metabolism, and vice versa, an effect of fatty acid metabolism on HPA-axis activity.

With regard to the effects of HPA-axis activity on fatty acid metabolism, it may be that the endophenotypic hypercortisolema observed in MDD-patients reduces synthesis and/or incorporation of unsaturated long chain fatty acids (e.g. DHA) in the cell membrane, resulting in altered fatty acid metabolism in MDD-patients. Indeed, decreased overall fatty acid peroxidizable, -unsaturation, and -chain length have been observed in MDD, with lower concentrations of the LCPUFAs EPA, DHA and total omega-3 fatty acids in blood and brain tissue. Initially, by decreasing membrane peroxidizable, these fatty acid changes may (possibly adaptively) protect against hypercortisolema induced oxidative stress. However, the involved fatty acids have important functional and structural roles in human (patho)physiology (e.g. cell membrane constituents, inflammatory and cytoarchitecture modulators). Hereby, the fatty acid alterations may affect (I) functioning of membrane-bound neurotransmitter receptors and gray and white matter integrity in the brain, and (II) triglyceride production, heart rate, myocardial efficiency, blood pressure, vascular resistance, endothelial dysfunction, and thrombosis, in the cardiovascular system.
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (N = 137)</th>
<th>Controls (N = 73)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, %</td>
<td>73.7%</td>
<td>69.9%</td>
<td>.55</td>
</tr>
<tr>
<td>Age, mean (SE), year</td>
<td>46.4 (0.8)</td>
<td>44.7 (1.1)</td>
<td>.205</td>
</tr>
<tr>
<td>Educational level&lt;sup&gt;a&lt;/sup&gt;, %</td>
<td></td>
<td></td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Low</td>
<td>33.3</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>31.2</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>35.5</td>
<td>72.3</td>
<td></td>
</tr>
<tr>
<td>Social class&lt;sup&gt;b&lt;/sup&gt;, %</td>
<td></td>
<td></td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Class 1</td>
<td>11.1</td>
<td>55.0</td>
<td></td>
</tr>
<tr>
<td>Class 2</td>
<td>52.1</td>
<td>32.0</td>
<td></td>
</tr>
<tr>
<td>Class 3</td>
<td>36.7</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>Smoking, %</td>
<td>49.0</td>
<td>39.4</td>
<td>.21</td>
</tr>
<tr>
<td>Body mass index, mean (SE), kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>26.0 (0.44)</td>
<td>24.8 (0.43)</td>
<td>.062</td>
</tr>
<tr>
<td>HDRS&lt;sup&gt;17&lt;/sup&gt; score, mean (SE)</td>
<td>5.9 (.46)</td>
<td>1.2 (.48)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Current depressive episode, % (n)</td>
<td>19.0 (26)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Antidepressant use, % (n)</td>
<td>62.8 (86)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>TCA, % (n)</td>
<td>5.1 (7)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>SSRI, % (n)</td>
<td>38.7 (53)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>SNRI, % (n)</td>
<td>11.7 (16)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Lithium and SNRI or TCA, % (n)</td>
<td>3.0 (4)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Lithium, % (n)</td>
<td>0.7 (1)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Other, % (n)</td>
<td>3.7 (5)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Number of previous episodes, mean (SE)</td>
<td>7.71 (0.76)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Age of first onset, mean (SE), year</td>
<td>28.4 (1.08)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>0800 h cortisol&lt;sup&gt;c,d&lt;/sup&gt;, mean (SE), nmol/L</td>
<td>3.70 (0.06)</td>
<td>3.44 (.08)</td>
<td>.009</td>
</tr>
<tr>
<td>2200 h cortisol&lt;sup&gt;d&lt;/sup&gt;, mean (SE), nmol/L</td>
<td>1.19 (0.07)</td>
<td>.50 (.09)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>EPA, mean (SE), pmol/10&lt;sup&gt;6&lt;/sup&gt; red blood cells</td>
<td>3.35 (0.14)</td>
<td>3.91 (.23)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>DHA, mean (SE), pmol/10&lt;sup&gt;6&lt;/sup&gt; red blood cells</td>
<td>14.92 (0.45)</td>
<td>20.20 (.75)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>AA, mean (SE), pmol/10&lt;sup&gt;6&lt;/sup&gt; red blood cells</td>
<td>71.96 (0.74)</td>
<td>81.33 (1.0)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>UI, mean (SE)</td>
<td>1.29 (0.01)</td>
<td>1.39 (.01)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>CLI, mean (SE)</td>
<td>18.32 (0.02)</td>
<td>18.55 (.01)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>PI, mean (SE)</td>
<td>1.10 (0.01)</td>
<td>1.22 (.01)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Abbreviations: HDRS, Hamilton depression rating scale; TCA, tricyclic antidepressant; SSRI, selective serotonin reuptake inhibitor; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; UI, unsaturation index; CLI, chain length index; PI, peroxidation index. <sup>a</sup> Educational level is defined as: low, primary education or preparatory middle-level applied education; middle, higher general continued education or middle-level applied education; and high, preparatory scientific education, higher applied education, or scientific education. <sup>b</sup> Based on occupation: Class 1, e.g. cleaner; Class 2, e.g. nurse; Class 3, e.g. general manager. <sup>c</sup> Averaged value over two values on two consecutive days. <sup>d</sup> Log transformed.
Therefore, in the long term, these fatty acid changes might very well affect the nervous system [e.g. hippocampal neuronal cell structure and function and cardiovascular system (atherosclerosis)]. Thereby, these (patho)physiological mechanisms may explain part of the increased risk for recurrence and/or cardiovascular comorbidity in MDD.

With regard to the effects of fatty acid metabolism on HPA-axis activity, the observed reduced fatty acid unsaturation and chain length in MDD-patients may have altered HPA-axis feedback in MDD, due to their influence on glucocorticoid receptor and p-glycoprotein functioning. Depending on degree of unsaturation and chain length, fatty acids modulate binding of glucocorticoids to the glucocorticoid receptor, possible by inducing a conformational change. Moreover, fatty acids have been hypothesized to regulate activity of p-glycoprotein, which is responsible for transport of cortisol across the blood-brain barrier. Fatty acids have been hypothesized to regulate p-glycoprotein activity by modulating prostaglandin E2 concentrations. In addition, research into cancer drug resistance showed that PUFAs reduce gene expression, protein production and pump activity of p-glycoprotein. Therefore, reduced p-glycoprotein activity resulting from fatty acid alterations may lead to reduced transport of cortisol across the blood-brain barrier. This reduced cortisol transport to the brain may explain disturbances in HPA-axis feedback, which have been hypothesized to form the main pathophysiological mechanism underlying hypercortisolemia in MDD-patients. This way, fatty acid alterations could provide a partial explanation for the observed hypercortisolemic trait in recurrent MDD-patients.

Table 2. Associations between morning and evening cortisol concentrations and fatty acid concentrations and indices, with and without correction for several confounders, in 137 patients with recurrent depression (linear regression)

<table>
<thead>
<tr>
<th>HPA-axis</th>
<th>FA (N = 137)</th>
<th>Uncorrected</th>
<th>Correctedd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td>0800 h cortisolab</td>
<td>EPAc</td>
<td>0.230 (0.294)</td>
<td>.783</td>
</tr>
<tr>
<td></td>
<td>DHAc</td>
<td>0.071 (0.681)</td>
<td>.104</td>
</tr>
<tr>
<td></td>
<td>AAC</td>
<td>-0.031 (1.258)</td>
<td>-0.26</td>
</tr>
<tr>
<td></td>
<td>UI</td>
<td>-0.002 (0.010)</td>
<td>-0.21</td>
</tr>
<tr>
<td></td>
<td>CLI</td>
<td>-0.024 (0.026)</td>
<td>-0.917</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>-0.005 (0.013)</td>
<td>-0.359</td>
</tr>
<tr>
<td>2200 h cortisolb</td>
<td>EPAc</td>
<td>-0.234 (0.193)</td>
<td>-1.22</td>
</tr>
<tr>
<td></td>
<td>DHAc</td>
<td>-1.358 (0.499)</td>
<td>-2.72</td>
</tr>
<tr>
<td></td>
<td>AAC</td>
<td>0.677 (1.136)</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>UI</td>
<td>-0.021 (0.009)</td>
<td>-2.42</td>
</tr>
<tr>
<td></td>
<td>CLI</td>
<td>-0.060 (0.025)</td>
<td>-2.41</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>-0.029 (0.012)</td>
<td>-2.48</td>
</tr>
</tbody>
</table>

Abbreviations: HDRS, Hamilton depression rating scale; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; UI, unsaturation index; CLI, chain length index; PI, peroxidation index. Bold numbers indicate statistical significance. a Average over two values on two consecutive days. b nmol/L, log transformed. c pmol/10^6 red blood cells. d Corrected for sex, age, marital status, educational level, social class, HDRS score, body mass index, smoking, antidepressant use, and depressive state.
These two directions of association provide three possible causal mechanisms as explanation for the relationship between HPA-axis activity and fatty acid metabolism, as observed in this study. First, the effect of the HPA-axis on fatty acid metabolism could be the primary cause of the relationship. Alternatively, the relationship could be explained by the effect of fatty acid metabolism on the HPA-axis. Finally, effects could operate in both ways, i.e. a bidirectional relationship, given that hypercortisolemia has been reported to induce these specific fatty acid disturbances,\textsuperscript{28,34,36,38} which on their turn can influence HPA-axis hyperactivity.\textsuperscript{42,46} In order to fully grasp the role of the delicate balance between these two systems in homeostasis and pathophysiology, further studies are warranted, preferably using repeated measurements of cortisol and fatty acid metabolism during randomized experimental interventions aimed at the HPA-axis or fatty acid metabolism in selected clinical samples (e.g. recurrent MDD patients).

**Figure 1.** Relationship between cortisol and docosahexaenoic acid concentrations in 137 patients with recurrent depression. Lines represent linear fit lines and 95% confidence intervals, $\beta$ (SE) = -1.358 (0.499), $t = -2.72$, $P = .006$. 

Cortisol 22:00, nmol/L, log transformed

Docosahexaenoic acid, pmol/10e6 erythrocytes
Interestingly, our results do not show a significant relationship between morning cortisol concentrations and fatty acid metabolism. This was also found in a study in healthy men, in which evening cortisol was more pronouncedly associated with metabolic effects than morning cortisol. However, our morning cortisol values are based on two subsequent morning values independent of awakening time, instead of multiple morning measures reflecting the cortisol awakening response. Therefore, it is possible that our sampling methods caused exogenous variability in the data, which could consequently serve as an explanation for the absence of an association between morning cortisol and fatty acid metabolism. Nonetheless, it can also be argued that increased evening cortisol concentrations (and the more negative associations with fatty acid metabolism) reflect the main pathophysiological HPA-axis disturbance in MDD, i.e. higher baseline HPA-axis activity. Indeed, differences in cortisol concentrations between patients and controls in our study were more pronounced in the evening than in the morning. On the other hand, although morning cortisol concentrations were higher compared to controls, relatively lower morning cortisol concentrations predicted recurrence in a patient group remitted from recurrent MDD. A similar pattern was also seen in a recent study which showed that in subjects who had been exposed to early life stress, relative hypercortisolemia was associated with less psychological distress, while a blunted cortisol response was associated with recurrent psychological distress. This could suggest that hypercortisolemia in a

Table 3. Associations between morning and evening cortisol concentrations and fatty acid concentrations and indices, with and without correction for several confounders, in 73 non-depressed controls (linear regression)

<table>
<thead>
<tr>
<th>HPA-axis</th>
<th>FA (N = 73)</th>
<th>Uncorrected</th>
<th>Corrected&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β (SE)</td>
<td>t</td>
</tr>
<tr>
<td>0800 h cortisol&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>EPA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.085 (0.373)</td>
<td>.228</td>
</tr>
<tr>
<td></td>
<td>DHA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.461 (1.202)</td>
<td>-.383</td>
</tr>
<tr>
<td></td>
<td>AA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.168 (1.588)</td>
<td>-1.37</td>
</tr>
<tr>
<td></td>
<td>UI</td>
<td>-0.001 (0.009)</td>
<td>-0.14</td>
</tr>
<tr>
<td></td>
<td>CLI</td>
<td>-0.004 (0.018)</td>
<td>-.194</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>-0.008 (0.014)</td>
<td>-.597</td>
</tr>
<tr>
<td>2200 h cortisol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>EPA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.152 (0.324)</td>
<td>-.470</td>
</tr>
<tr>
<td></td>
<td>DHA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.207 (1.034)</td>
<td>.200</td>
</tr>
<tr>
<td></td>
<td>AA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.351 (1.410)</td>
<td>.249</td>
</tr>
<tr>
<td></td>
<td>UI</td>
<td>0.007 (0.008)</td>
<td>.866</td>
</tr>
<tr>
<td></td>
<td>CLI</td>
<td>0.011 (0.017)</td>
<td>.666</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>0.009 (0.012)</td>
<td>.717</td>
</tr>
</tbody>
</table>

Abbreviations: HDRS, Hamilton depression rating scale; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; UI, unsaturation index; CLI, chain length index; PI, peroxidation index. <sup>a</sup> Average over two values on two consecutive days. <sup>b</sup> nmol/L, log transformed. <sup>c</sup> pmol/10<sup>6</sup> red blood cells. <sup>d</sup> Corrected for sex, age, marital status, educational level, social class, HDRS<sub>17</sub>-score, body mass index and smoking.
subgroup of patients (particularly those who experienced early life stress) might represent an adaptive phenomenon, possibly preventive against MDD-recurrence (e.g. on the shorter-term). However, this could be at the cost of hypercortisolemia associated perturbations in fatty acid metabolism, potentially inducing elevated allostatic load associated cardiovascular risk (e.g. on the longer-term). In conclusion, the relevance of the mechanisms underlying the differences in associations of morning versus evening cortisol concentrations with fatty acid metabolism remains to be further elucidated.

Some limitations need to be addressed further. First, the observed relations could be a reflection of confounding factors. Despite correction for e.g. educational level, social class, anthropometric characteristics and smoking, we did not correct for diet. The interest in the relationship between MDD and fatty acid metabolism initially began with the observation of a negative relationship between fatty fish intake and MDD prevalence.62 This may indicate that diet could have had a confounding influence in our study. However, more recently, some studies suggest relatively small dietary influences on fatty acid concentrations,24,64 as a result of strict endogenous regulation. Furthermore, because of the associations between demographic and anthropometric factors and diet, correction for these factors may have already reduced the influence of diet. Finally, stress is associated with increased dietary preference for high-caloric, palatable foods, which contain relatively more saturated fatty acid and less LCPUFAs. This may possibly alter physiological fatty acid concentrations, in which case diet may be considered as a mediator of the relationship between the HPA-axis and fatty acid metabolism, instead of a confounder.65,66 Interestingly, as a potential adaptive mechanism, this altered dietary preference seems to attenuate HPA-axis activity and associated behavior.65 However, the initially observed correlations between fish intake and MDD were relatively strong; therefore, further studies are needed to investigate whether dietary preference acts as a confounder and/or a mediator of the observed relation between HPA-axis activity and fatty acid metabolism.

Second, although we used multiple imputation to reduce bias introduced by missing values, it could still have been that missing values influenced our result. Multiple imputation is based upon the assumption that data are missing at random. This means that missingness (i.e. whether data are missing or not) may depend on observed data, but not on unobserved data.52 In our study, we were able to include several predictors in the imputation model (e.g. psychopathological, demographic, and other biological variables), which increases the change that missingness is accounted for by observed data. In addition, most missing data in our study is missing completely at random, e.g. due to laboratory or logistic accidents, which in any case would not result in biases.51 Furthermore, bias by missing values would not easily explain the observed relations. Comparing results with and without multiple imputation should be done with caution. In general, in the present study, effect parameters without imputation had similar directions as those after multiple imputation, with only modest differences between results with and without imputation for individual fatty acids (Supplementary discussion). However, replication of our findings would strengthen the evidence.
Third, fatty acid analyses were performed on concentrations of fatty acids from different subclasses combined, without differentiation with regard to phospholipid class, e.g. phosphatidylcholine, sphingomyelin. This differentiation could have produced more distinguished results and would be an interesting addition in further research. Furthermore, future research could benefit from inclusion of cholesterol concentrations in the analyses, given the interplay between the HPA-axis, fatty acid metabolism, and cholesterol.\textsuperscript{3,31}

Fourth, sleep quality and time of awaking were not included in the model, which could have influenced HPA-axis activity and fatty acid metabolism.\textsuperscript{67} Finally, no measures of HPA-axis feedback (e.g. dexamethasone suppression test) were included in the present study. Although the used methodology have been previously shown to be able to adequately quantify differences in HPA-axis activity both between patients and controls and different patient subclasses,\textsuperscript{16} it would be an interesting option for future research to link alterations in fatty acid metabolism to measures of HPA-axis feedback, especially considering the suggested association of fatty acid metabolism with p-glycoprotein functioning.\textsuperscript{47}

### Table 4.

Cortisol (morning, evening) by group [patients, $N = 137$; controls, $N = 73$ (reference category)] interaction-terms explaining fatty acid concentrations and indices, with and without correction for confounders (linear regression)

<table>
<thead>
<tr>
<th>HPA-axis</th>
<th>FA</th>
<th>Uncorrected</th>
<th>Corrected\textsuperscript{a}</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>t</td>
<td>P</td>
<td>β (SE)</td>
</tr>
<tr>
<td>0800 h cortisol\textsuperscript{a,b}</td>
<td>EPA\textsuperscript{c}</td>
<td>0.146 (0.473)</td>
<td>.308</td>
<td>.760</td>
</tr>
<tr>
<td></td>
<td>DHA\textsuperscript{c}</td>
<td>0.531 (1.238)</td>
<td>.429</td>
<td>.668</td>
</tr>
<tr>
<td></td>
<td>AA\textsuperscript{c}</td>
<td>-2.199 (2.057)</td>
<td>-1.07</td>
<td>.287</td>
</tr>
<tr>
<td></td>
<td>UI</td>
<td>-0.001 (0.015)</td>
<td>-.049</td>
<td>.961</td>
</tr>
<tr>
<td></td>
<td>CLI</td>
<td>-0.020 (0.039)</td>
<td>-.520</td>
<td>.603</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>0.004 (0.021)</td>
<td>.174</td>
<td>.862</td>
</tr>
<tr>
<td>2200 h cortisol\textsuperscript{b}</td>
<td>EPA\textsuperscript{c}</td>
<td>-0.082 (0.341)</td>
<td>-.240</td>
<td>.811</td>
</tr>
<tr>
<td></td>
<td>DHA\textsuperscript{c}</td>
<td>-1.565 (1.026)</td>
<td>-1.53</td>
<td>.127</td>
</tr>
<tr>
<td></td>
<td>AA\textsuperscript{c}</td>
<td>0.326 (1.813)</td>
<td>.180</td>
<td>.858</td>
</tr>
<tr>
<td></td>
<td>UI</td>
<td>-0.028 (0.013)</td>
<td>-2.25</td>
<td>.024</td>
</tr>
<tr>
<td></td>
<td>CLI</td>
<td>-0.071 (0.033)</td>
<td>-2.17</td>
<td>.030</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>-0.038 (0.017)</td>
<td>-2.15</td>
<td>.032</td>
</tr>
</tbody>
</table>

**Abbreviations:** HDRS, Hamilton depression rating scale; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; UI, unsaturation index; CLI, chain length index; PI, peroxidation index. Bold numbers indicate statistical significance. \textsuperscript{a} Average over two values on two consecutive days. \textsuperscript{b} nmol/L, log transformed. \textsuperscript{c} pmol/10\textsuperscript{6} red blood cells. \textsuperscript{d} Corrected for sex, age, marital status, educational level, social class, HDRS\textsubscript{17}-score, body mass index and smoking.
Strengths of our study are the inclusion of highly recurrent depressed patients as representatives of a more biologically determined MDD-subtype, which may be specifically linked to recurrence and cardiovascular co-morbidity. Therefore, the included patient group can be considered characteristic for those patients particularly causing the large MDD-associated burden of disease. In addition, our study allowed for the investigation of the relationship between cortisol and fatty acid metabolism in MDD-patients in comparison with matched controls. Our findings suggest HPA-axis and fatty acid metabolism interactions as a (patho)physiological mechanism underlying MDD recurrence and its association with cardiovascular disease, which first need replication. Thereafter, our findings could provide new targets for treatments to prevent recurrence in MDD.

In conclusion, our results corroborate that HPA-axis activity is associated with fatty acid metabolism in recurrent MDD. More specifically, evening cortisol concentrations are significantly negatively associated with fatty acid indices in recurrent MDD-patients relative to controls. Future randomized experimental intervention studies using clinical outcome measures could help to further elucidate the suggested effects of hypercortisolemia in the brain and cardiovascular system in recurrent MDD.

![Figure 2. Relationships between cortisol and the unsaturation index (UI), chain length index (CLI) and peroxidation index (PI) compared between 137 recurrently depressed patients and 73 controls. Lines represent linear fit lines and 95% confidence intervals. For UI: $\beta$ (SE) = -0.028 (0.013), $t$ = -2.25, $P = .024$; for CLI: $\beta$ (SE) = -0.071 (0.033), $t$ = -2.17, $P = .030$; for PI: $\beta$ (SE) = -0.038 (0.017), $t$ = -2.15, $P = .032$.](image-url)
ROLE OF FUNDING SOURCE

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CONFLICTS OF INTEREST

All authors report no biomedical financial interests or potential conflicts of interest.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.01.013.

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