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

Mocking, Roel J T; Ruhe, Eric; Assies, Johanna; Lok, Anja; Koeter, Maarten W J; Visser, Ieke; Bockting, Claudi L H; Schene, Aart H

*Published in:*
Psychoneuroendocrinology

*DOI:*
10.1016/j.psyneuen.2013.01.013



*Publication date:*
2013

Psychoneuroendocrinology (2013) 38, 1607—1617

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

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

Roel J.T. Mocking[a,*], Henricus G. Ruhé[a], Johanna Assies[a], Anja Lok[a], Maarten W.J. Koeter[a], Ieke Visser[a], Claudi L.H. Bockting[b], Aart H. Schene[a]

[a] *Program for Mood Disorders, Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands*
[b] *Department of Clinical Psychology, University of Groningen, Groningen, The Netherlands*

Received 8 October 2012; received in revised form 14 January 2013; accepted 14 January 2013

**KEYWORDS**
Psychiatry;
Depressive disorder;
Major;
Glucocorticoids;
Cortisol;
Recurrence;
Fatty acids;
n-3 PUFA;
Eicosapentaenoic acid;
Docosahexaenoic acids;
Metabolic networks and pathways

**Summary** Alterations in hypothalamic—pituitary—adrenal (HPA)-axis activity and fatty acid (FA)-metabolism have been observed in (recurrent) major depressive disorder (MDD). Through the pathophysiological roles of FAs in the brain and cardiovascular system, a hypothesized relationship between HPA-axis activity and FA-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 FA-metabolism measured in the red blood cell membrane: (I) three main FAs [eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA)], and (II) structural FA indices (unsaturation, chain length, peroxidation) calculated from concentrations of 29 FAs to delineate overall FA-characteristics. In addition, we compared these associations in patients with those in controls.

In patients, evening cortisol concentrations were significantly negatively associated with DHA ($B = -1.358$; 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 ($B = -0.060$; SE = 0.025; $t = -2.41$; $p = .019$), and peroxidation index ($B = -0.029$; SE = 0.012; $t = -2.48$; $p = .015$). The relations between cortisol and the latter three variables were significantly negative in patients relative to controls. Significance remained after correction for confounders.

Our results suggest a relationship between HPA-axis activity and FA-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.


* Corresponding author at: Department of Psychiatry, Academic Medical Centre, Meibergdreef 5, Amsterdam 1105 AZ, The Netherlands. Tel.: +31 208913694.
*E-mail address:* R.J.Mocking@amc.uva.nl (R.J.T. Mocking).

0306-4530/$ — see front matter 
http://dx.doi.org/10.1016/j.psyneuen.2013.01.013

## 1. Introduction

Major depressive disorder (MDD) accounts for an overwhelming global burden of disease. This is mainly due to its (I) lifelong recurrent nature (Greden, 2001), and (II) association with cardiovascular comorbidity (Charlson et al., 2011). 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 (FA)-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 (Stetler and Miller, 2011; Herbert, 2012), both during MDD-episodes and in remission, suggesting an endophenotype (Lok et al., 2011).

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 (Goodyer et al., 2000), as well as recurrent episodes in remitted MDD-patients (Appelhof et al., 2006), possibly through the effects of excess cortisol on the brain, particularly the hippocampus (Sapolsky, 2000; Kronmüller et al., 2008; Ursache et al., 2012). However, mixed results have also been observed (Bockting et al., 2012). In addition, hypercortisolemia is predictive of prospective death from cardiovascular disease in MDD-patients (Jokinen and Nordström, 2009). 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 FA-metabolism has been consistently reported in MDD (Assies et al., 2010; Lin et al., 2010; Yager et al., 2010), both in acutely depressed and remitted patients (Assies et al., 2010). Main findings are lower concentrations of ω3 long chain polyunsaturated fatty acids (LCPUFA) [e.g. eicosapentaenoic acid (C20:5ω3; EPA) and docosahexaenoic acid (C20:6ω3; DHA)] (Assies et al., 2010; Lin et al., 2010), and decreased overall FA unsaturation, chain length and peroxidizability (Mocking et al., 2012b).

FAs have important structural and functional (patho)physiological roles in both the nervous and cardiovascular system (Piomelli et al., 2007; Mozaffarian and Wu, 2011; McNamara, in press; Samieri et al., 2012). Structurally, FAs are major components of (neuronal) membranes (Piomelli et al., 2007). Unsaturation and chain length of membrane FAs determine membrane fluidity, which on its turn influences functioning of membrane bound proteins, e.g. neurotransmitter receptors and cardiac ion channels (Piomelli et al., 2007). Moreover, membrane FA peroxidizability determines membrane susceptibility to oxidative stress (Mocking et al., 2012b). Functionally, FAs [particularly EPA, DHA and arachidonic acid (C20:4ω6; AA)] are involved in inflammatory regulation (Hibbeln and Salem, 1995; Mozaffarian and Wu, 2011), and maintenance of brain cytoarchitecture (Rao et al., 2006; McNamara, in press).

Previous studies have found a modulating effect of HPA-axis activity on FA-metabolism. Cortisol influences mobilization (Conner et al., 1996; Brenner et al., 2001; Macfarlane et al., 2008), lipolysis (Brenner et al., 2001), oxidation (Hibbeln and Salem, 1995; Flerov et al., 2003), and synthesis (Hibbeln and Salem, 1995; Brenner et al., 2001) of FAs. For example, cortisol inhibits Δ5- and Δ6-desaturase-activity (de Alaniz and Marra, 2003), enzymes responsible for unsaturation of FA chains. In addition, oxidative stress associated with hypercortisolemia (Sato et al., 2010) could influence FA concentrations (Hibbeln and Salem, 1995; Flerov et al., 2003; Yager et al., 2010). These influences seem to have differential effects on specific FAs (Hibbeln and Salem, 1995; Conner et al., 1996; de Alaniz and Marra, 2003; Gounarides et al., 2008), in such a way that high cortisol concentrations are associated with a decrease in ω3 LCPUFA concentrations and FA unsaturation, chain length and peroxidizability.

Vice versa, FAs also seem to affect the HPA-axis (Lanfranco et al., 2004). Dietary supplementation of ω3 LCPUFA (e.g. EPA) reduced cortisol concentrations in rats (Song et al., 2003), healthy subjects (Delarue et al., 2003), and MDD-patients (Jazayeri et al., 2010; Mocking et al., 2012a). In addition, a maternal preweaning ω3 PUFA deficient diet induces HPA-axis hyperactivity in rat offspring (Chen and Su, 2012). Furthermore, in chronically stressed monkeys, the ω6/ω3 ratio was positively associated with cortisol response to acute stress (Laugero et al., 2011). Supplementation of ω3 LCPUFA increases concentrations of EPA and DHA (polyunsaturated FAs with a long chain length), and decreases concentrations of AA. These FA-alterations may alter the feedback of the HPA-axis in three ways: (I) FAs influence glucocorticoid receptor functioning, depending on their degree of unsaturation and chain length (Vallette et al., 1991), (II) EPA and AA modulate p-glycoprotein function and thereby cortisol transport across the blood–brain barrier (Murck et al., 2004), 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) (Hibbeln and Salem, 1995; Schiepers et al., 2005).

Based on this literature, a relationship between the HPA-axis and FA-metabolism can be expected. Through the effects of FAs 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 FA-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 FA-metabolism in MDD by testing the associations between cortisol and FA-concentrations in patients with recurrent MDD and matched controls. We hypothesized that cortisol would be negatively associated with (I) concentrations of ω3 LCPUFAs (e.g. EPA and DHA), and (II) indicators of overall FA-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.

## 2. Methods

### 2.1. Participants

Recruitment of the study population has been described in more detail previously (Assies et al., 2010; Lok et al., 2011). 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 vs. naturalistic care on recurrence in patients with recurrent MDD. Inclusion criteria of the original trial were: $\geq$2 previous MDD-episodes in the last 5 years, as defined by the Structured Clinical Interview for DSM-IV disorders (SCID) (First et al., 1996); in remission >10 weeks and <2 years, as defined by a score < 10 on the 17-item Hamilton Depression Rating Scale ($HDRS_{17}$) (Hamilton, 1960); 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 (Greden, 2001). We did not exclude patients based on depressive status or medication (e.g. antidepressants) use, because previously, both FA-metabolism and HPA-axis activity were not substantially influenced by these factors in this population (Assies et al., 2010; Lok et al., 2011).

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.

### 2.2. 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 (Lok et al., 2011). We measured depressive symptoms in all subjects using the $HDRS_{17}$ (Hamilton, 1960).

#### 2.2.1. HPA-axis

For the HPA-axis, we measured concentrations of cortisol, expressed in nmol/ml, 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) (Lok et al., 2011), 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 (Lok et al., 2011). Both averaged morning and evening cortisol were normally distributed after log transformation.

#### 2.2.2. FA-metabolism

We used washed erythrocyte FA-concentrations as a model of brain FA-concentrations. From blood, sampled in the non-fasting state, we separated and washed erythrocytes and stored them at −80 °C until analyses by capillary gas chromatography, as described previously (Assies et al., 2010). We expressed concentrations of 29 different FAs in pmol/$10^6$ erythrocytes. To analyze the association between HPA-axis activity and overall FA-metabolism, we calculated three indices that delineate main structural FA-characteristics on the basis of all 29 FA-concentrations (Mocking et al., 2012b). The (I) unsaturation index (UI) denotes the mean number of double bounds per FA; (II) chain length index (CLI), provides information about the mean number of carbon atoms per FA; and (III) peroxidation index (PI), delineates the mean FA susceptibility to oxidative stress.

### 2.3. Statistical analyses

To (I) prevent bias possibly introduced by missing values, and (II) facilitate calculation of indices – despite non-detectable FA in individual patients – and thereby remaining adequate power, we used multiple imputation, as described previously (Lok et al., 2011; Mocking et al., 2012b). 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 (Donders et al., 2006). 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 (Graham, 2009).

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 (Lok et al., 2011; Mocking et al., 2012b), 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, $HDRS_{17}$ score, weight, length, waist and hip circumference, smoking, and salivary cortisol and dehydroepiandrosterone sulphate, folic acid, vitamin $B_6$ and $B_{12}$, homocysteine, and all other measured FA-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 FA concentration must lie between 0.001 and the detection limit of that FA (with 99% confidence) (Mocking et al., 2012b). 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 FA or index as outcome variable. We corrected for confounders, selected a priori, by adding relevant variables (sex, age, marital status, educational level, social class, $HDRS_{17}$-score, BMI and smoking) as predictors to the model. In addition, although FA- and cortisol concentrations were not substantially influenced by antidepressant use or depressive state in this population (Assies et al., 2010; Lok et al., 2011), 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—FA-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 FA-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.

## 3. Results

### 3.1. 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 FA-metabolism, reflected by both lower concentrations of EPA, DHA and AA and lower FA-indices (Assies et al., 2010; Lok et al., 2011; Mocking et al., 2012b).

### 3.2. Association between HPA-axis and FAs in patients

Table 2 shows results of linear regression models of associations between morning and evening cortisol and FA-concentrations and -indices in patients. All indices and DHA (Fig. 1) were negatively associated with evening cortisol concentrations ($.006 \leq p \leq .019$); significance remained after correction for possible confounders ($.003 \leq p \leq .023$), and after exclusion of TCA users. This indicates that more pronounced evening hypercortisolemia was associated with lower concentrations of the ω3 LCPUFA DHA and decreased overall FA unsaturation, chain length and peroxidizability. Morning cortisol concentrations were not significantly associated with FA-metabolism. Results without multiple imputation are provided in Table S1.

### 3.3. Association between HPA-axis and FAs in controls

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

### 3.4. Differences between patients and controls in the association between HPA-axis and FAs

The group × evening cortisol interaction-term was significant for the UI, CLI and PI ($.024 \leq p \leq .032$; Table 4; Fig. 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 FA unsaturation, chain length and peroxidizability in patients relative to controls. These differences remained after correction for confounders ($.034 \leq p \leq .049$). Results remained significant after exclusion of TCA users, except for the corrected effect of the group × evening cortisol interaction regarding the PI ($p = .063$). The group × evening cortisol interaction-term was not significant for the individual FA-concentrations ($p > .127$). The group × morning cortisol interaction was not significant for any of the indicators of FA-metabolism. Results without multiple imputation are provided in Table S3.

## 4. Discussion

In this study, we investigate the relationship between HPA-axis activity and FA-metabolism in recurrent MDD by testing associations between cortisol and FA-concentrations in 137 patients with ≥2 previous MDD-episodes and comparing them

**Table 1** Subject characteristics.

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

*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.

[a] 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.

[b] Based on occupation: Class 1, e.g. cleaner; Class 2, e.g. nurse; Class 3, e.g. general manager.

[c] Averaged value over two values on two consecutive days.

[d] Log transformed.

with 73 matched controls. Consistent with our hypotheses, evening cortisol concentrations were significantly negatively associated with the ω3 LCPUFA DHA, and overall FA unsaturation, chain length and peroxidizability in the patients. Furthermore, we found that the associations of evening cortisol with FA 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, FA-unsaturation, chain length and peroxidizability underline the relationship between functioning of the HPA-axis and FA-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 (Hibbeln et al., 2004), and 5α-dihydroprogesterone in patients diagnosed with either alcohol abuse, MDD, or both (Nieminen et al., 2010).

Studies that experimentally influenced this relationship between HPA-axis activity and FA-metabolism by administration of FAs or glucocorticoids suggest that effects could operate in two directions (Hibbeln and Salem, 1995; Delarue et al., 2003): on the one hand, an effect of the HPA-axis on FA-metabolism, and vice versa, an effect of FA-metabolism on HPA-axis activity.

With regard to the effects of HPA-axis activity on FA-metabolism, it may be that the endophenotypic hypercortisolemia observed in MDD-patients reduces synthesis and/or incorporation of unsaturated long chain FAs (e.g. DHA) in the cell membrane (Hibbeln and Salem, 1995; Brenner et al., 2001; Flerov et al., 2003), resulting in altered FA metabolism in MDD-patients. Indeed, decreased overall FA-peroxidizability, -unsaturation, and -chain length have been observed in MDD, with lower concentrations of the LCPUFAs EPA, DHA and total ω3 FAs in blood and brain tissue (Assies et al., 2010; Lin et al., 2010; McNamara, 2010; Yager et al., 2010; Mocking et al., 2012b). Initially, by decreasing membrane peroxidiz-

**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).

| HPA-axis | FA ($n$ = 137) | Uncorrected | | | Corrected[d] | | |
|---|---|---|---|---|---|---|---|
| | | $B$ (SE) | $t$ | $p$ | $B$ (SE) | $t$ | $p$ |
| 0800 h cortisol[a,b] | EPA[c] | 0.230 (0.294) | .783 | .446 | 0.020 (0.235) | .085 | .932 |
| | DHA[c] | 0.071 (0.681) | .104 | .918 | −0.444 (0.697) | −.637 | .526 |
| | AA[c] | −0.031 (1.258) | −.024 | .981 | −0.096 (1.524) | −.063 | .950 |
| | UI | −0.002 (0.010) | −0.21 | .837 | −0.010 (0.010) | −.938 | .349 |
| | CLI | −0.024 (0.026) | −.917 | .359 | −0.037 (0.028) | −1.35 | .178 |
| | PI | −0.005 (0.013) | −.359 | .720 | −0.017 (0.014) | −1.23 | .220 |
| 2200 h cortisol[b] | EPA[c] | −0.234 (0.193) | −1.22 | .225 | −0.308 (0.177) | −1.74 | .083 |
| | DHA[c] | −1.358 (0.499) | −2.72 | **.006** | −1.567 (0.524) | −2.99 | **.003** |
| | AA[c] | 0.677 (1.136) | 0.60 | .555 | 0.469 (1.189) | .395 | .696 |
| | UI | −0.021 (0.009) | −2.42 | **.018** | −0.022 (0.009) | −2.34 | **.022** |
| | CLI | −0.060 (0.025) | −2.41 | **.019** | −0.062 (0.024) | −2.55 | **.012** |
| | PI | −0.029 (0.012) | −2.48 | **.015** | −0.029 (0.012) | −2.34 | **.023** |

*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/ml, log transformed.

[c] pmol/$10^6$ erythrocytes.

[d] Corrected for sex, age, marital status, educational level, social class, $HDRS_{17}$-score, body mass index, smoking, antidepressant use, and depressive state.

ability, these FA-changes may (possibly adaptatively) protect against hypercortisolemia induced oxidative stress. However, the involved FAs have important functional and structural roles in human (patho)physiology [e.g. cell membrane constituents (Piomelli et al., 2007; McNamara, in press), inflammatory (Hibbeln and Salem, 1995) and cytoarchitecture modulators (Rao et al., 2006)]. Hereby, the FA-alterations may affect (I) functioning of membrane-bound neurotransmitter receptors and gray and white matter integrity in the brain (Piomelli et al., 2007; McNamara, in press); and (II) triglyceride production, heart rate, myocardial efficiency, blood pressure, vascular resistance, endothelial dysfunction, and thrombosis, in the cardiovascular system (Mozaffarian and Wu, 2011). Therefore, in the long term, these FA-changes might very well affect the nervous [e.g. hippocampal neuronal cell structure and function (Sapolsky, 2000; McNamara, in press; Samieri et al., 2012)] and cardiovascular system (atherosclerosis) (Yager et al., 2010; Mozaffarian and Wu, 2011). Thereby, these (patho)physiological mechanisms may explain part of the increased risk for recurrence and/or cardiovascular comorbidity in MDD (Harris, 2007; Kronmüller et al., 2008; Jokinen and Nordström, 2009; Yager et al., 2010; Harris et al., in press; McNamara, in press; Ursache et al., 2012).

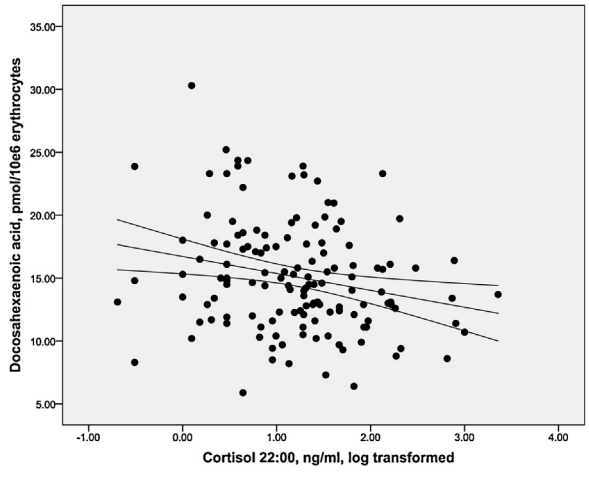


**Figure 1** Relationship between cortisol and docosahexaenoic acid concentrations in 137 patients with recurrent depression. Lines represent linear fit lines and 95% confidence intervals. $B$ (SE) = −1.358 (0.499), $t$ = −2.72, $p$ = .006.

With regard to the effects of FA-metabolism on HPA-axis activity, the observed reduced FA 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 (Vallette et al., 1991; Murck et al., 2004). Depending on degree of unsaturation and chain length, FA modulate binding of glucocorticoids to the glucocorticoid receptor, possible by inducing a conformational change (Vallette et al., 1991; Sumida, 1995). Moreover, FAs have been hypothesized to regulate activity of p-glycoprotein, which is responsible for transport of cortisol across the blood–brain barrier. FAs have been hypothesized to regulate p-glycoprotein activity by modulating prostaglandin E2 concentrations (Murck et al., 2004). In addition, research into cancer drug resistance showed that PUFAs reduce gene expression, protein production and pump activity of p-glycoprotein (Kuan et al., 2011). Therefore, reduced p-glycoprotein activity resulting from FA-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 (Herbert, 2012). This way, FA-alterations could provide 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).

| HPA-axis | FA ($n = 73$) | Uncorrected | | | Corrected[d] | | |
|---|---|---|---|---|---|---|---|
| | | *B* (SE) | *t* | *p* | *B* (SE) | *t* | *p* |
| 0800 h cortisol[a,b] | EPA[c] | 0.085 (0.373) | .228 | .820 | −0.262 (0.416) | −.631 | .530 |
| | DHA[c] | −0.461 (1.202) | −.383 | .702 | −0.878 (1.358) | −.646 | .518 |
| | AA[c] | 2.168 (1.588) | −1.37 | .172 | 3.165 (1.669) | 1.90 | .059 |
| | UI | −0.001 (0.009) | −0.14 | .886 | −0.001 (0.010) | −.116 | .908 |
| | CLI | −0.004 (0.018) | −.194 | .846 | −0.004 (0.021) | −.173 | .863 |
| | PI | −0.008 (0.014) | −.597 | .550 | −0.010 (0.015) | −.646 | .518 |
| 2200 h cortisol[b] | EPA[c] | −0.152 (0.324) | −.470 | .639 | −0.232 (0.327) | −.709 | .478 |
| | DHA[c] | 0.207 (1.034) | .200 | .841 | −0.022 (1.141) | −.019 | .985 |
| | AA[c] | 0.351 (1.410) | .249 | .803 | 0.755 (1.600) | .472 | .638 |
| | UI | 0.007 (0.008) | .866 | .386 | 0.006 (0.009) | .658 | .511 |
| | CLI | 0.011 (0.017) | .666 | .507 | 0.009 (0.020) | .481 | .632 |
| | PI | 0.009 (0.012) | .717 | .474 | 0.006 (0.013) | .414 | .679 |

*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.
[a] Average over two values on two consecutive days.
[b] nmol/ml, log transformed.
[c] pmol/$10^6$ erythrocytes.
[d] Corrected for sex, age, marital status, educational level, social class, $HDRS_{17}$-score, body mass index and smoking.

partial explanation for the observed hypercortisolemic trait in recurrent MDD-patients.

These two directions of association provide three possible causal mechanisms as explanation for the relationship between HPA-axis activity and FA-metabolism, as observed in this study. First, the effect of the HPA-axis on FA-metabolism could be the primary cause of the relationship. Alternatively, the relationship could be explained by the effect of FA-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 FA-disturbances (Brenner et al., 2001; Flerov et al., 2003; Piomelli et al., 2007; Sato et al., 2010), which on their turn can influence HPA-axis hyperactivity (Vallette et al., 1991; Delarue et al., 2003). 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

**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).

| HPA-axis | FA | Uncorrected | | | Corrected[d] | | |
|---|---|---|---|---|---|---|---|
| | | *B* (SE) | *t* | *p* | *B* (SE) | *t* | *p* |
| 0800 h cortisol[a,b] | EPA[c] | 0.146 (0.473) | .308 | .760 | 0.178 (0.411) | .432 | .666 |
| | DHA[c] | 0.531 (1.238) | .429 | .668 | 0.355 (1.205) | .295 | .768 |
| | AA[c] | −2.199 (2.057) | −1.07 | .287 | −1.937 (2.099) | −.923 | .358 |
| | UI | −0.001 (0.015) | −.049 | .961 | −0.005 (0.016) | −.331 | .741 |
| | CLI | −0.020 (0.039) | −.520 | .603 | −0.023 (0.040) | −.579 | .563 |
| | PI | 0.004 (0.021) | .174 | .862 | −0.003 (0.022) | −.126 | .900 |
| 2200 h cortisol[b] | EPA[c] | −0.082 (0.341) | −.240 | .811 | −0.002 (0.316) | −.007 | .994 |
| | DHA[c] | −1.565 (1.026) | −1.53 | .127 | −1.530 (1.013) | −1.51 | .131 |
| | AA[c] | 0.326 (1.813) | .180 | .858 | −0.085 (1.808) | −0.047 | .963 |
| | UI | −0.028 (0.013) | −2.25 | **.024** | −0.027 (0.013) | −2.12 | **.035** |
| | CLI | −0.071 (0.033) | −2.17 | **.030** | −0.070 (0.033) | −2.13 | **.034** |
| | PI | −0.038 (0.017) | −2.15 | **.032** | −0.035 (0.018) | −1.97 | **.049** |

*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/ml, log transformed.
[c] pmol/$10^6$ erythrocytes.
[d] Corrected for sex, age, marital status, educational level, social class, $HDRS_{17}$-score, body mass index and smoking.

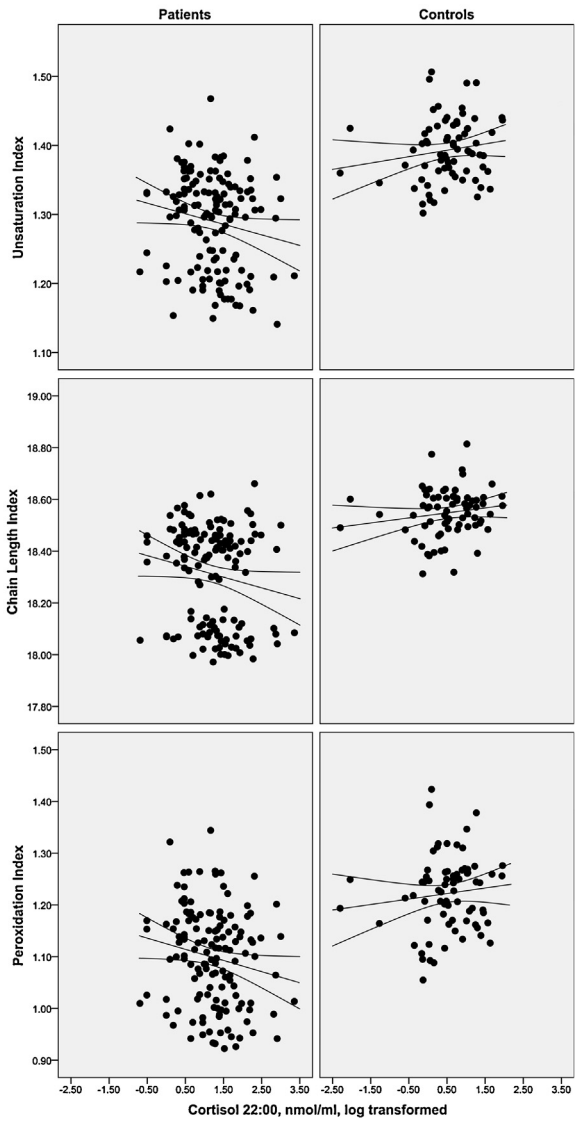


**Figure 2** Relationships between cortisol and the unsaturation index (UI), chain length index (CLI) and peroxidation index (PI) compared between 137 recurrently depressed patients and 73 controls. Lines represent linear fit lines and 95% confidence intervals. For UI: *B* (SE) = −0.028 (0.013), $t = -2.25$, $p = .024$; for CLI: *B* (SE) = −0.071 (0.033), $t = -2.17$, $p = .030$; for PI: *B* (SE) = −0.038 (0.017), $t = -2.15$, $p = .032$.

FA-metabolism during randomized experimental interventions aimed at the HPA-axis or FA-metabolism in selected clinical samples (e.g. recurrent MDD patients).

Interestingly, our results do not show a significant relationship between morning cortisol concentrations and FA-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 (Plat et al., 1999). 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 FA-metabolism. Nonetheless, it can also be argued that increased evening cortisol concentrations (and the more negative associations with FA-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 (Bockting et al., 2012). 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 (Goldman-Mellor et al., 2012). This could suggest that hypercortisolemia in a 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 FA-metabolism, potentially inducing elevated allostatic load associated cardiovascular risk (e.g. on the longer-term; Fries et al., 2005). In conclusion, the relevance of the mechanisms underlying the differences in associations of morning versus evening cortisol concentrations with FA-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 FA-metabolism initially began with the observation of a negative relationship between fatty fish intake and MDD prevalence (Appleton et al., 2008). 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 FA-concentrations (Assies et al., 2010; Bentsen et al., 2011), 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 FA and less LCPUFAs. This may possibly alter physiological FA-concentrations, in which case diet may be considered as a mediator of the relationship between the HPA-axis and FA-metabolism, instead of a confounder (Dallman et al., 2003; Groesz et al., 2012). Interestingly, as a potential adaptive mechanism, this altered dietary preference seems to attenuate HPA-axis activity and associated behavior (Dallman et al., 2003). 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 FA-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 (Graham, 2009). 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 (Donders et al., 2006). 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 FAs (Supplementary discussion). However, replication of our findings would strengthen the evidence.

Third, FA-analyses were performed on concentrations of FAs 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, FA-metabolism, and cholesterol (Hibbeln and Salem, 1995; Mocking et al., 2012a). Fourth, sleep quality and time of awaking were not included in the model, which could have influenced HPA-axis activity and FA-metabolism (Irmisch et al., 2007). 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 (Lok et al., 2011), it would be an interesting option for future research to link alterations in FA-metabolism to measures of HPA-axis feedback, especially considering the suggested association of FA-metabolism with p-glycoprotein functioning (Murck et al., 2004).

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 FA-metabolism in MDD-patients in comparison with matched controls. Our findings suggest HPA-axis and FA-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 FA-metabolism in recurrent MDD. More specifically, evening cortisol concentrations are significantly negatively associated with FA 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.

## Role of funding source

Funding support for this study was provided by The Netherlands Foundation for Mental Health, Utrecht, the Netherlands organization for health research and development Prevention Program (ZonMw), and an AMC PhD Scholarship from the Graduate School of the Academic Medical Center, University of Amsterdam. The study's funding sources had no role in the study design; collection, analysis, and interpretation of data; writing of the report; and decision to submit the paper for publication. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

## Conflicts of interest

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

## Acknowledgments

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

## Appendix A. 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.

## References

de Alaniz, M.J.T., Marra, C.A., 2003. Steroid hormones and fatty acid desaturases. Prostaglandins Leukot. Essent. Fatty Acids 68, 163—170.

Appelhof, B.C., Huyser, J., Verweij, M., Brouwer, J.P., van Dyck, R., Fliers, E., Hoogendijk, W.J., Tijssen, J.G., Wiersinga, W.M., Schene, A.H., 2006. Glucocorticoids and relapse of major depression (dexamethasone/corticotropin-releasing hormone test in relation to relapse of major depression). Biol. Psychiatry 59, 696—701.

Appleton, K.M., Rogers, P.J., Ness, A.R., 2008. Is there a role for n-3 long-chain polyunsaturated fatty acids in the regulation of mood and behaviour?. A review of the evidence to date from epidemiological studies, clinical studies and intervention trials. Nutr. Res. Rev. 21, 13—41.

Assies, J., Pouwer, F., Lok, A., Mocking, R.J., Bockting, C.L., Visser, I., Abeling, N.G., Duran, M., Schene, A.H., 2010. Plasma and erythrocyte fatty acid patterns in patients with recurrent depression: a matched case—control study. PLoS ONE 5, e10635.

Bentsen, H., Solberg, D.K., Refsum, H., Gran, J.M., Bøhmer, T., Torjesen, P.A., Halvorsen, O., Lingjærde, O., 2011. Bimodal distribution of polyunsaturated fatty acids in schizophrenia suggests two endophenotypes of the disorder. Biol. Psychiatry 70, 97—105.

Bockting, C.L., Lok, A., Visser, I., Assies, J., Koeter, M.W., Schene, A.H., 2012. Lower cortisol levels predict recurrence in remitted patients with recurrent depression: a 5.5 year prospective study. Psychiatry Res. 200, 281—287.

Brenner, R.R., Ayala, S., Garda, H.A., 2001. Effect of dexamethasone on the fatty acid composition of total liver microsomal lipids and phosphatidylcholine molecular species. Lipids 36, 1337—1345.

Charlson, F., Stapelberg, N., Baxter, A., Whiteford, H., 2011. Should global burden of disease estimates include depression as a risk factor for coronary heart disease? BMC Med. 9, 47.

Chen, H.F., Su, H.M., 2012. Exposure to a maternal n-3 fatty acid-deficient diet during brain development provokes excessive hypothalamic—pituitary—adrenal axis responses to stress and behavioral indices of depression and anxiety in male rat offspring later in life. J. Nutr. Biochem. 24, 70—80.

Conner, W.E., Lin, D.S., Colvis, C., 1996. Differential mobilization of fatty acids from adipose tissue. J. Lipid Res. 37, 290—298.

Dallman, M.F., Pecoraro, N., Akana, S.F., La Fleur, S.E., Gomez, F., Houshyar, H., Bell, M.E., Bhatnagar, S., Laugero, K.D., Manalo, S., 2003. Chronic stress and obesity: a new view of ''comfort food''. Proc. Natl. Acad. Sci. U.S.A. 100, 11696—11701.

Delarue, J., Matzinger, O., Binnert, C., Schneiter, P., Chiolero, R., Tappy, L., 2003. Fish oil prevents the adrenal activation elicited by mental stress in healthy men. Diabetes Metab. 29, 289—295.

Donders, A.R., van der Heijden, G.J., Stijnen, T., Moons, K.G., 2006. Review: a gentle introduction to imputation of missing values. J. Clin. Epidemiol. 59, 1087—1091.

First, M.B., Gibbon, M., Spitzer, R.L., Williams, J.B., 1996. User Guide for the Structured Clinical Interview for DSM-IV Axis-1 Disorders. American Psychiatric Association, Washington, DC.

Flerov, M.A., Gerasimova, I.A., Rakitskaya, V.V., 2003. Lipid peroxidation in the striatum of rats during stress after administration of cortisol. Neurosci. Behav. Physiol. 33, 889—891.

Fries, E., Hesse, J., Hellhammer, J., Hellhammer, D.H., 2005. A new view on hypocortisolism. Psychoneuroendocrinology 30, 1010—1016.

Goldman-Mellor, S., Hamer, M., Steptoe, A., 2012. Early-life stress and recurrent psychological distress over the lifecourse predict divergent cortisol reactivity patterns in adulthood. Psychoneuroendocrinology 37, 1755—1768.

Goodyer, I.M., Tamplin, A., Herbert, J., Altham, P.M.E., 2000. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. Br. J. Psychiatry 177, 499—504.

Gounarides, J.S., Korach-André, M., Killary, K., Argentieri, G., Turner, O., Laurent, D., 2008. Effect of dexamethasone on glucose tolerance and fat metabolism in a diet-induced obesity mouse model. Endocrinology 149, 758—766.

Graham, J.W., 2009. Missing data analysis: making it work in the real world. Annu. Rev. Psychol. 60, 549—576.

Greden, J.F., 2001. The burden of recurrent depression: causes, consequences, and future prospects. J. Clin. Psychiatry 62 (Suppl.), 5—9.

Groesz, L.M., McCoy, S., Carl, J., Saslow, L., Stewart, J., Adler, N., Laraia, B., Epel, E., 2012. What is eating you? Stress and the drive to eat. Appetite 58, 717—721.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry 23, 56—62.

Harris, W.S., 2007. Omega-3 fatty acids and cardiovascular disease: a case for omega-3 index as a new risk factor. Pharmacol. Res. 55, 217—223.

Harris, W.S., Kennedy, K.F., O'Keefe, J.H., Jr, Spertus, J.A. Red blood cell fatty acid levels improve GRACE score prediction of 2-yr mortality in patients with myocardial infarction. Int. J. Cardiol., in press.

Herbert, J., 2012. Cortisol and depression: three questions for psychiatry. Psychol. Med. 8, 1—21.

Hibbeln, J.R., Salem, N., 1995. Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. Am. J. Clin. Nutr. 62, 1—9.

Hibbeln, J.R., Bissette, G., Umhau, J.C., George, D.T., 2004. Omega-3 status and cerebrospinal fluid corticotrophin releasing hormone in perpetrators of domestic violence. Biol. Psychiatry 56, 895—897.

Irmisch, G., Schläfke, D., Gierow, W., Herpertz, S., Richter, J., 2007. Fatty acids and sleep in depressed inpatients. Prostaglandins Leukot. Essent. Fatty Acids 76, 1—7.

Jazayeri, S., Keshavarz, S.A., Tehrani-Doost, M., Djalali, M., Hosseini, M., Amini, H., Chamari, M., Djazayery, A., 2010. Effects of eicosapentaenoic acid and fluoxetine on plasma cortisol, serum interleukin-1beta and interleukin-6 concentrations in patients with major depressive disorder. Psychiatry Res. 178, 112—115.

Jokinen, J., Nordström, P., 2009. HPA axis hyperactivity and cardiovascular mortality in mood disorder inpatients. J. Affect. Disord. 116, 88—92.

Kronmüller, K.T., Pantel, J., Köhler, S., Victor, D., Giesel, F., Magnotta, V.A., Mundt, C., Essig, M., Schröder, J., 2008. Hippocampal volume and 2-year outcome in depression. Br. J. Psych. 192, 472—473.

Kuan, C.Y., Walker, T.H., Luo, P.G., Chen, C.F., 2011. Long-chain polyunsaturated fatty acids promote paclitaxel cytotoxicity via inhibition of the MDR1 gene in the human colon cancer Caco-2 cell line. J. Am. Coll. Nutr. 30, 265—273.

Lanfranco, F., Giordano, R., Pellegrino, M., Gianotti, L., Ramunni, J., Picu, A., Baldi, M., Ghigo, E., Arvat, E., 2004. Free fatty acids exert an inhibitory effect on adrenocorticotropin and cortisol secretion in humans. J. Clin. Endocrinol. Metab. 89, 1385—1390.

Laugero, K.D., Smilowitz, J.T., German, J.B., Jarcho, M.R., Mendoza, S.P., Bales, K.L., 2011. Plasma omega 3 polyunsaturated fatty acid status and monounsaturated fatty acids are altered by chronic social stress and predict endocrine responses to acute stress in titi monkeys. Prostaglandins Leukot. Essent. Fatty Acids 84, 71—78.

Lin, P.Y., Huang, S.Y., Su, K.P., 2010. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. Biol. Psychiatry 68, 140—147.

Lok, A., Mocking, R.J., Ruhé, H.G., Visser, I., Koeter, M.W., Assies, J., Bockting, C.L., Olff, M., Schene, A.H., 2011. Longitudinal hypothalamic—pituitary—adrenal axis trait and state effects in recurrent depression. Psychoneuroendocrinology 37, 892—902.

Macfarlane, D.P., Forbes, S., Walker, B.R., 2008. Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome. J. Endocrinol. 197, 189—204.

McNamara, R.K., 2010. DHA deficiency and prefrontal cortex neuropathology in recurrent affective disorders. J. Nutr. 40, 864—868.

McNamara, R.K. Deciphering the role of docosahexaenoic acid in brain maturation and pathology with magnetic resonance imaging. Prostaglandins Leukot. Essent. Fatty Acids, in press.

Mocking, R.J., Assies, J., Bot, M., Jansen, E.H., Schene, A.H., Pouwer, F., 2012a. Biological effects of add-on eicosapentaenoic acid supplementation in diabetes mellitus and co-morbid depression: a randomized controlled trial. PLoS ONE 7 (11), e49431, http://dx.doi.org/10.1371/journal.pone.0049431.

Mocking, R.J., Assies, J., Lok, A., Ruhé, H.G., Koeter, M.W., Visser, I., Bockting, C.L., Schene, A.H., 2012b. Statistical methodological issues in handling of fatty acid data: percentage or concentration, imputation and indices. Lipids 47, 541—547.

Mozaffarian, D., Wu, J.H., 2011. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J. Am. Coll. Cardiol. 58, 2047—2067.

Murck, H., Song, C., Horrobin, D.F., Uhr, M., 2004. Ethyl-eicosapentaenoate and dexamethasone resistance in therapy-refractory depression. Int. J. Neuropsychopharmacol. 7, 341—349.

Nieminen, L.R.G., Makino, K.K., Mehta, N., Virkkunen, M., Kim, H.Y., Hibbeln, J.R., 2010. Relationship between omega-3 fatty acids and plasma neuroactive steroids in alcoholism, depression and controls. Prostaglandins Leukot. Essent. Fatty Acids 75, 309—314.

Piomelli, D., Astarita, G., Rapaka, R., 2007. A neuroscientist's guide to lipidomics. Nat. Rev. Neurosci. 8, 743—754.

Plat, L., Leproult, R., L'Hermite-Baleriaux, M., Fery, F., Mockel, J., Polonsky, K.S., Van Cauter, E., 1999. Metabolic effects of short-term elevations of plasma cortisol are more pronounced in the evening than in the morning. J. Clin. Endocrinol. Metab. 84, 3082—3092.

Rao, J.S., Ertley, R.N., Lee, H.J., DeMar Jr., J.C., Arnold, J.T., Rapoport, S.I., Bazinet, R.P., 2006. n-3 Polyunsaturated fatty acid deprivation in rats decreases frontal cortex BDNF via a p38 MAPK-dependent mechanism. Mol. Psychiatry 12, 36—46.

Samieri, C., Maillard, P., Crivello, F., Proust-Lima, C., Peuchant, E., Helmer, C., Amieva, H., Allard, M., Dartigues, J.F., Cunnane, S.C., Mazoyer, B.M., Barberger-Gateau, P., 2012. Plasma long-chain omega-3 fatty acids and atrophy of the medial temporal lobe. Neurology 79, 642—650.

Sapolsky, R.M., 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch. Gen. Psychiatry 57, 925—935.

Sato, H., Takahashi, T., Sumitani, K., Takatsu, H., Urano, S., 2010. Glucocorticoid generates ROS to induce oxidative injury in the hippocampus, leading to impairment of cognitive function of rats. J. Clin. Biochem. Nutr. 47, 224—232.

Schiepers, O.J.G., Wichers, M.C., Maes, M., 2005. Cytokines and major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 29, 201—217.

Song, C., Phillips, A.G., Leonard, B.E., Horrobin, D.F., 2003. Ethyl-eicosapentaenoic acid ingestion prevents corticosterone-mediated memory impairment induced by central administration of interleukin-1beta in rats. Mol. Psychiatry 9, 630—638.

Stetler, C., Miller, G.E., 2011. Depression and hypothalamic—pituitary—adrenal activation: a quantitative summary of four decades of research. Psychosom. Med. 73, 114—126.

Sumida, C., 1995. Fatty acids: ancestral ligands and modern co-regulators of the steroid hormone receptor cell signalling pathway. Prostaglandins Leukot. Essent. Fatty Acids 52, 137—144.

Ursache, A., Wedin, W., Tirsi, A., Convit, A., 2012. Preliminary evidence for obesity and elevations in fasting insulin mediating associations between cortisol awakening response and hippocampal volumes and frontal atrophy. Psychoneuroendocrinology 37, 1270—1276.

Vallette, G.E.N.E., Vanet, A.N.N.E., Sumida, C.H.A.R., Nunez, E.A., 1991. Modulatory effects of unsaturated fatty acids on the binding of glucocorticoids to rat liver glucocorticoid receptors. Endocrinology 129, 1363—1369.

Yager, S., Forlenza, M.J., Miller, G.E., 2010. Depression and oxidative damage to lipids. Psychoneuroendocrinology 35, 1356—1362.