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Erythrocyte fatty acid profiles and plasma homocysteine, folate and vitamin B_6 and B_{12} in recurrent depression: Implications for co-morbidity with cardiovascular disease

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ABSTRACT

Oxidative stress induced interactions between fatty acid (FA) and one-carbon metabolism may be involved in co-occurrence of major depressive disorder (MDD) and cardiovascular disease (CVD), which have been scarcely studied together. In 137 recurrent MDD-patients vs. 73 age- and sex-matched healthy controls, we simultaneously measured key components of one-carbon metabolism in plasma (homocysteine, folate, vitamins B_6 and B_{12}), and of FA-metabolism in red blood cell membranes [main polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA) and structural FA-indices (chain length, unsaturation, peroxidation)]. Results show significant positive associations of folate with EPA, DHA, and the peroxidation index, which were similar in patients and controls. After correction for confounders, these associations were lost except for EPA. Associations between B-vitamins and FA-parameters were non-significant, but also similar in patients and controls. Homocysteine and DHA were significantly less negatively associated in patients than in controls. In conclusion, these data indicate similarities but also differences in associations between parameters of one-carbon and FA-metabolism in recurrent MDD patients vs. controls, which may reflect differences in handling of oxidative stress. Further research should test the consequences of these differences, particularly the premature development of CVD in MDD.

1. Introduction

Persons with CVD have a 2.4 fold increased risk for MDD, while persons with MDD are at increased risk to develop CVD (McIntyre et al., 2009; Goldbacher et al., 2009; Kahl et al., 2012). Although the mechanisms that link both diseases are still unclear, there is increasing evidence for a fundamental role of oxidative stress (Ng et al., 2008; Roberts and Sind, 2009; Schiavone et al., 2012, Assies et al., 2014), which might be a common denominator of MDD and CVD through its influence on the shared biochemistry of fatty acid (FA) and one-carbon metabolisms. Thereby oxidative stress may modulate signaling and functioning of cell types particularly relevant to the pathogenesis of both CVD and MDD such as neurons, endothelial cells, immune cells and platelets (Severus et al., 2001; Assies et al., 2014).

Regarding FA-metabolism, FAs have important structural and functional (patho)physiological roles in both the nervous and cardiovascular system (Piomelli et al., 2007; McNamara, 2009). Structurally, FAs are key components of (neuronal and vascular) cell membranes (Piomelli et al., 2007). Unsaturation and chain length of membrane FAs determine membrane fluidity, which in turn influences functioning of membrane bound proteins, e.g. neurotransmitter receptors and cardiac ion channels.. Moreover,

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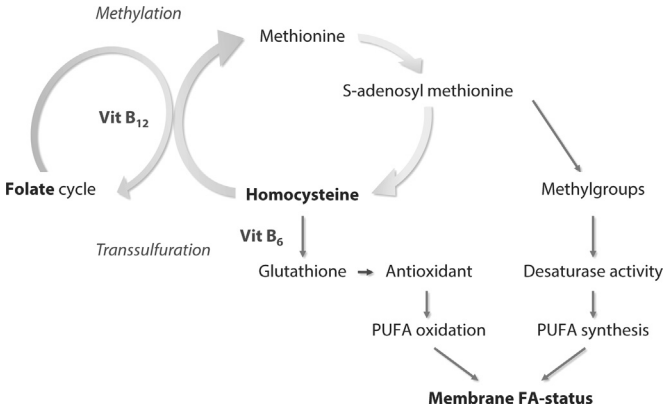

Fig. 1. Biological interplay between one-carbon and fatty acid metabolism. *Abbreviations*: Vit, vitamin; PUFA, polyunsaturated fatty acid. The one-carbon metabolism is closely linked to fatty acid metabolism in various ways. First, through the one-carbon-cycle, methyl groups from folate are delivered via S-adenosyl methionine to influence desaturase activity which is essential for PUFA synthesis. Second, using vitamin B_6, homocysteine can be converted to glutathione, which is a major antioxidant and thereby can prevent PUFA oxidation.

oxidative stress susceptibility is determined by FA-peroxidizability, which has also been found to be lower in MDD-patients (Hulbert et al., 2007; Mocking et al., 2012a). Functionally, FAs [particularly polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA)] and their (non)enzymatic peroxidation products are increasingly shown to be fundamentally involved in regulation of oxidative stress, inflammation, and brain cytoarchitecture maintenance (Mocking et al., 2012a; Terlecky et al., 2012; Baek and Park, 2013; McNamara, 2013). Interestingly, MDD and CVD have each been consistently associated with corresponding alterations in FA-metabolism: both MDD and CVD patients have lower omega-3 (n-3) long chain PUFAs (especially EPA, C20:5n-3 and DHA, C22:6n-3) and (n-3):(n-6) PUFA ratios in plasma and erythrocytes (Assies et al., 2010; Lin et al., 2010; Mozzafarian and Wu, 2011; Assies et al., 2014).

Regarding the one-carbon metabolism (Fig. 1), homocysteine is a key intermediate and indicator of systemic oxidative stress levels (Stanger et al., 2009; Hofmann, 2011). In the *transmethylation pathway*, homocysteine is transformed to S-adenosylmethionine – a universal donor of methyl groups – with vitamin B_{12} and folate as co-factors. Methylgroups are essential for epigenetic regulation of DNA-transcription (McGowan et al., 2008). In the *transsulfuration pathway*, homocysteine is condensed to the principle cellular antioxidant glutathione, with vitamin B_6 as co-factor (Forman et al., 2009) (Fig. 1). MDD and CVD are also associated with comparable oxidative stress related alterations in one-carbon metabolism, such as high homocysteine and low folate levels (Bjelland et al., 2003; Morris et al., 2003; Bottiglieri, 2005; Kim et al., 2008; Humphrey et al., 2008; Murakami et al., 2008; Stanger et al., 2009; Wang et al., 2012; Nabi et al., 2013; Lok et al., 2014) (Fig. 1).

Of note, one-carbon- and FA-metabolism interact (Fig. 1). Biochemically, methyl groups from one-carbon metabolism are used for various steps in FA-transport and -synthesis, e.g. desaturase and elongase activity regulation. Methylgroups are also used for the synthesis of phosphatidylcholine critical for the delivery of important PUFAs from the liver to the plasma and peripheral tissues (Devlin & Green, 2009). Vice versa, FAs may influence one-carbon metabolism by e.g. effects on oxidative stress and expression of genes involved in homocysteine synthesis (Berstad et al., 2007).

Human observational research reported an inverse association of DHA but not for EPA with homocysteine and a positive association with folate, in various non-psychiatric, healthy and metabolically diseased populations (Li et al., 2006, 2007; Huang et al., 2012; Kume et al., 2013; Huang et al., 2013). Intervention studies showed that folate administration in rats increased n-3 PUFA (EPA, DHA) in plasma and tissue lipids, but AA was unaffected (Pita and Delgado, 2000).

In humans, lowering plasma homocysteine using folate, vitamin B_{12}, and vitamin B_6 had no effect on plasma n-3 long-chain PUFA (Crowe et al., 2008), while short-term vitamin B_6 restriction decreased plasma n-3 and n-6 PUFA concentrations and tended to increase the plasma (n-3):(n-6) PUFA ratio (Zhao et al., 2012). The other way around, n-3 PUFA supplementation induced significant decreases in plasma homocysteine, this effect was dose dependent and non-linear (Piolot et al., 2003; Zeman et al., 2006; Pooya et al., 2010; Huang et al., 2011).

Severus et al. (2001) first proposed the importance of the link between one-carbon metabolism and FA-metabolism in psychiatric disorders, but to our knowledge, only three studies have investigated this relation. The first, an uncontrolled explorative study of our group in 44 patients with (recurrent) MDD, reported a decrease in n-3 PUFAs in erythrocyte membranes and a significant positive association between the sum of n-6 PUFAs and plasma homocysteine (Assies et al., 2004). The second, a study in never-medicated patients with schizophrenia, showed that erythrocyte membrane DHA reductions paralleled significant increases in plasma homocysteine (Kale et al., 2010). The third, an intervention study of our group, showed that add-on EPA-supplementation did not affect one-carbon metabolites in diabetes mellitus patients with comorbid MDD (Mocking et al., 2012b).

We now extended our earlier pilot study to 137 recurrent MDD-patients, and additionally included 73 matched non-depressed controls. Although we already reported alterations in FA-metabolism and one-carbon metabolism in these patients compared to controls (Assies et al., 2010; Lok et al., 2014) we did not examine possible differences in nature and strength of associations of the parameters of FA- and one-carbon metabolism between patients and controls.

We hypothesized that in recurrent MDD-patients (I) homocysteine would be negatively associated with EPA, DHA, FA-chain length, -unsaturation, and -peroxidizability, (II) folate, vitamin B_6, and vitamin B_{12} would be positively associated with EPA, DHA, FA-chain length, -unsaturation, and -peroxidizability. In addition, given the above proposed altered handling of oxidative stress, we expected these relations to be more outspoken in recurrent MDD-patients.

2. Methods and materials

2.1. Study subjects

2.1.1. Enrollment and diagnosis

The present study was an add-on to a randomized controlled trial investigating the effect of cognitive therapy on recurrence in patients with recurrent MDD (Bockting et al., 2005; Lok et al., 2011). At two years follow-up of the trial, we invited participating patients for this add-on study. In addition, we recruited controls through media-advertisements, matched using strata based on gender and 5-year age groups. 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.

2.1.2. Inclusion criteria

Both patients and controls had to be aged 18–65. For the patients, inclusion criteria of the initial trial were: at least 2 previous MDD-episodes in the last 5 years, according to the Structured Clinical Interview for DSM-IV disorders (SCID) (First et al., 1996);

in remission for more than 10 weeks and less than 2 years, as defined by a score lower than 9 on the 17-item Hamilton Depression Rating Scale ($HDRS_{17}$) (Hamilton, 1960).

2.1.3. Exclusion criteria

Exclusion criteria for both patients and controls 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. In addition, 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. For the current study at two years follow-up of the initial trial, current depressive state or medication (e.g. antidepressants) use formed no exclusion criteria, because these factors have been previously shown to have no significant influence on FA-metabolism (and the one-carbon cycle) in this sample (Assies et al., 2010; Lok et al., 2014). So, the current study included both depressed/remitted and unmedicated/medicated recurrently depressed subjects.

2.2. Measures

2.2.1. Clinical measures

At two years follow-up of the initial trial, we asked subjects for marital status, educational level, social class, smoking behavior, and assessed their body mass index (BMI; weight/length^2). We measured depressive symptoms in all subjects with the $HDRS_{17}$ (Hamilton,1960).

2.2.2. Biochemical estimations

At the same two years follow-up, we collected blood samples by venipuncture in the non-fasting state at patient's home; control subjects came to the hospital for logistic reasons.

2.2.2.1. FA analysis. We measured erythrocyte FA-concentrations as a reflection of brain FA-concentrations (Carver et al., 2001; Guest et al., 2013). We separated and washed erythrocytes and stored them at −80 °C until capillary gas chromatography analysis, as described previously (Assies et al., 2010). We expressed concentrations of 29 different FAs in pmol/10^6 erythrocytes, including EPA, DHA, and AA. To analyze the associations between one-carbon metabolites and overall FA-metabolism, we calculated three indices that delineate main structural FA-characteristics on the basis of all 29 FA-concentrations): (I) The 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 (Mocking et al., 2012a). In detail, the UI is calculated as follows: (1 × monoenoics + 2 × dienoics + 3 × trienoics + 4 × tetraenoics - + 5 × pentaenoics + 6 × hexaenoics)/total FA-concentration; the CLI by adding the products of each FA's concentration and the number of carbon atoms in its carbon chain and dividing this with the total FA-concentration; and the PI as (0.025 × monoenoics + 1 × dienoics + 2 × trienoics + 4 × tetraenoics + 6 × pentaenoics + 8 × hexaenoics)/total FA-concentration.

2.2.2.2. Homocysteine, folate, vitamins B_6 and B_{12}. Within 4 h of collection, we separated plasma homocysteine and stored it at −80 °C until analysis. Storage duration did not exceed several weeks. Serum folate and vitamin B_6 and B_{12} were measured by immunoassay. Using isocratic high-performance liquid chromatography (HPLC) electrospray tandem mass-spectrometry, we determined homocysteine. Intra- and interassay coefficients of variation were linear in range from 2–150 μmol/L, and within 3.6% and 4%, respectively.

2.3. Analyses

2.3.1. Data handling

Due to technical reasons, as described earlier (Lok et al., 2014), no values could be obtained for respectively: folate ($n=7$), vitamin B6 ($n=7$), vitamin B12 ($n=5$), and homocysteine ($n=15$) of the 210 conceivable total samples. In addition, as also described earlier (Mocking et al., 2013), 14.3%, 13.8% and 14.3% of EPA, DHA and AA values were missing or non-detectable, respectively. To prevent bias possibly introduced by missing values we used multiple imputation using package Amelia II, as described previously (Donders et al., 2006; Graham, 2009; Mocking et al., 2012a). In the imputation procedure, we included independent and dependent variables of our statistical regression models (see below), and other variables that correlated with these variables, e.g. other biological parameters. This resulted in 5 imputed data sets on which we performed subsequent analyses; for which we pooled results. Standard diagnostics of Amelia II suggested successful multiple imputation. Vitamin and homocysteine concentrations resembled normal distributions after log transformations, which were used in the analyses throughout, except for vitamin B_{12}.

2.3.2. Statistics

Analyses were performed with Statistical Package for the Social Sciences (SPSS) version 22. Patients' and controls' baseline characteristics were compared using χ^2 and Student's *t*-test statistics. We tested the associations between one-carbon metabolites and FA-metabolism parameters using multiple regression analyses. For our first hypothesis (the relationship between one-carbon and FA-metabolism in patients) we used multiple regression models with a one-carbon-metabolite as predictor and a FA-parameter as outcome variable in the patient group. We corrected for potential confounders (age, sex, education, socioeconomic status, marital status) by including them as additional predictors in the models. In addition, we tested the effect of waist circumference (reflecting the metabolic syndrome) as a factor on the causal path from oxidative stress to one-carbon and FA-metabolism alterations. For our second hypothesis (on differences in the relationships between one-carbon and FA-metabolism between patients and age- and sex-matched controls) we used multiple regression models in the combined sample (patients and controls) with as predictors group (patients vs. controls), the selected one-carbon-metabolite concentration, and an interaction term (group × metabolite), and as dependent variable a given FA-parameter. If the interaction term contributed significantly to the model, we concluded that the relationship between the one-carbon-metabolite and FA-parameter was significantly modified by group, i.e. the relationship differed between patients and controls. Again, we corrected for confounders (education, socioeconomic status, smoking, marital status, and also waist circumference) by adding them as predictors to the models.

3. Results

3.1. Participant characteristics (Table 1)

One hundred and thirty-seven patients and 73 controls participated. Matching was successful for age and sex; patients differed on some demographic variables (lower educational level and social class in the patient group). Most patients were recovered (81%) from depression while one in five was depressed (SCID) at the time of sampling. FAs and one-carbon metabolites were not correlated with depressive symptoms measured with the HDRS (all *P*'s > .257). During sampling, 62.8% used antidepressants, of which selective serotonin reuptake inhibitors (SSRIs) were the most

Table 1
Subject characteristics.

Characteristic	Patients (n=137)	Controls (n=73)	P value
Age, mean (SD), year	46.4 (9.5)	44.7 (9.4)	0.205
Educational level[a]			<0.001
Low, %	33.3	5.2	
Middle, %	31.2	22.5	
High, %	35.5	72.3	
Single, %	38.4	30.1	0.265
Social class[b]			<0.001
Class 1, %	55.0	11.2	
Class 2, %	32.0	52.1	
Class 3, %	13.0	36.7	
Smoking, %	39.4	49.0	0.211
BMI, mean (SD), kg/m^2	26.0 (4.8)	24.8 (3.5)	0.062
Waist circumference, mean (SD), cm	89.3 (13.9)	84.9 (12.1)	0.025
AD-use during sampling, %	62.8	NA	
Of which SSRIs, %	64.0	NA	
Received cognitive therapy, %	54	NA	
$HDRS_{17}$ score, mean (SD)	5.9	NA	
Number of previous episodes, mean (SD)	7.7	NA	
Depressed during sampling, %	19	NA	
Age of first onset, year	28.4	NA	
Psychiatric diseases first relatives (%)	68	NA	
Folate[c], mean (SE), nmol/L	3.12 (0.03)	3.23 (0.04)	0.025
Vitamin B_6[c], mean (SE), nmol/L	4.37 (0.05)	4.49 (0.07)	0.183
Vitamin B_{12}, mean (SE), pmol/L	314.1 (10.6)	304.0 (14.7)	0.580
Homocysteine[c], mean (SE), μmol/L	2.23 (0.03)	2.30 (0.04)	0.100
EPA, mean (SE), pmol/10^6 erythrocytes	3.35 (0.14)	3.91 (0.23)	<0.001
DHA, mean (SE), pmol/10^6 erythrocytes	14.92 (0.45)	20.20 (0.75)	<0.001
AA, mean (SE), pmol/10^6 erythrocytes	71.96 (0.74)	81.33 (1.0)	<0.001
Chain length index, mean (SE)	18.32 (0.01)	18.55 (0.01)	<0.001
Unsaturation index, mean (SE)	1.29 (0.01)	1.39 (0.01)	<0.001
Peroxidation index, mean (SE)	1.10 (0.01)	1.22 (0.01)	<0.001

Abbreviations: AD, antidepressant (missing n=5); BMI, body mass index; HDRS, Hamilton depression rating scale; SSRI, selective serotonin reuptake inhibitor.
We operationalized smoking dichotomously (yes/no).

[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] Social class is based on occupation: Class 1, e.g. cleaner; Class 2, e.g. nurse; Class 3, e.g. general manager. Group comparisons were calculated using Students-t tests, X^2 test of Fisher exact test.

[c] Log transformed.

commonly described antidepressants (64%).

Hypothesis I. Relationships between one-carbon metabolites and FA-parameters in recurrently depressed patients.

Relationship between folate and FA-metabolism in patients (Table 2, Fig. 2)

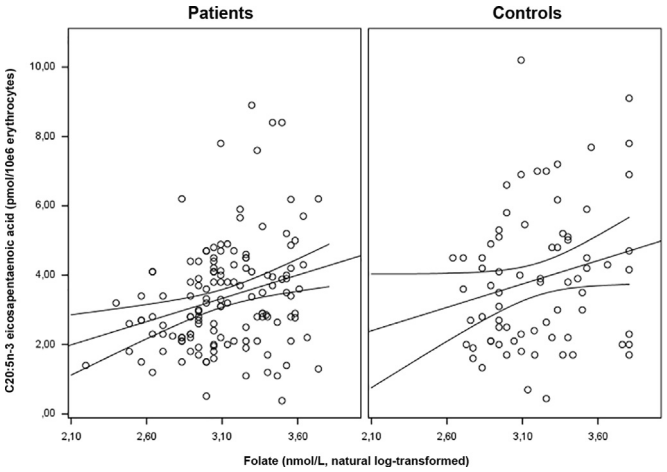

Fig. 2. Relation between plasma folate (nmol/L, natural log-transformed) and erythrocyte eicosapentaenoic acid (pmol/10e6 erythrocytes) in recurrently depressed patients (N=137) and matched controls (N=73).

In the recurrently depressed patient group, folate was significantly positively associated with EPA (P=0.002) and DHA (P=0.041). The positive association with the peroxidation index showed a trend (P=0.072). After correcting for confounding factors (age, sex, marital status, education, socioeconomic status) the positive association between folate and EPA remained (P=0.010). After additional correction for waist circumference, the association between folate and EPA was significant at trend level (P=.066).

Relationship between other one-carbon metabolites and FA-metabolism in patients (Table S1)

There were no significant correlations between homocysteine and vitamin B_6 and B_{12} and fatty acid concentrations and the chain length, unsaturation and peroxidation indices in recurrently depressed patients.

Hypothesis II. Differences in the relationships of one-carbon metabolites and FA-parameters between recurrently depressed patients and matched healthy controls.

Differences in the relations of folate and B-vitamins with FA metabolism between patients and controls (Table S2)

The group × metabolite interaction terms were not significant for folate, vitamin B_6 and B_{12}, indicating that the associations between FA-metabolism and folate, vitamin B_6 and B_{12}, did not differ between patients and controls, also not after correction for confounders.

Differences in relations between homocysteine and FA characteristics between patients and controls (Table 3,Fig. 3)

A significant interaction between group and homocysteine on DHA indicated that homocysteine was significantly less negatively associated with homocysteine in the patients compared to the controls (P=0.010). This difference in association between patients and controls persisted after correction for confounding factors (P=0.013).

Table 2
Relations between plasma folic acid (nmol/L, natural log-transformed) and erythrocyte fatty acid concentrations (pmol/10e6 erythrocytes) and the unsaturation, chain length and peroxidation indices in recurrently depressed patients (N=137).

Fatty acid	Beta	Std error	95% CI	t	Df	Correlation	P value
C20:5w3	1.303	0.429	0.461–2.144	3.035	136	0.256	**0.002**
C22:6w3	2.776	1.339	0.120–5.433	2.073	136	0.196	**0.041**
C20:4w6	−0.862	2.263	−5.299 to 3.576	−0.381	136	−0.033	0.703
Unsaturation index	0.028	0.021	−0.13 to 0.069	1.340	136	0.124	0.182
Chain length index	0.029	0.056	−0.82 to 0.140	0.512	136	0.047	0.609
Peroxidation index	0.051	0.028	−0.005 to 0.107	1.810	136	0.167	0.072

Table 3
Differences between recurrently depressed patients ($N=137$) and healthy controls ($N=73$) in relations of plasma homocysteine (μmol/L, natural log-transformed) with erythrocyte fatty acid concentrations (pmol/10e6 erythrocytes) and the unsaturation, chain length and peroxidation indices.[a]

Fatty acid	Beta Int	Std error	95% CI	*t*	Df	Correlation	*P* Value
C20:5w3	1.092	0.909	−0.696 to 2.879	1.200	209	0.088	0.231
C22:6w3	7.101	2.740	1.711–12.491	2.592	209	0.188	**0.010**
C20:4w6	0.246	4.559	−8.763 to 9.256	0.054	209	0.004	0.957
Unsaturation index	0.029	0.033	−0.36 to 0.094	0.864	209	0.062	0.388
Chain length index	0.077	0.090	−0.101 to 0.256	0.855	209	0.064	0.393
Peroxidation index	0.054	0.047	−0.038 to 0.146	1.158	209	0.084	0.247

[a] Displayed are the Beta values of the group (patients vs. controls) by homocysteine interaction terms (Beta Int) of the multiple regression models for each fatty acid parameter, and their corresponding statistical characteristics including *P* values. A significant Beta Int suggests a difference between patients and controls in the association of homocysteine and the given fatty acid parameter (see also Fig. 3). Bold values indicate statistical significance ($P<.05$).

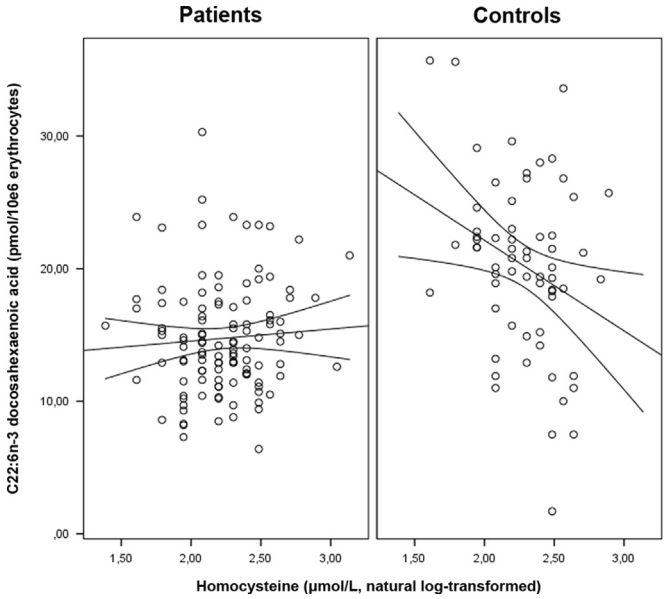

Fig. 3. Differences between recurrently depressed patients ($N=137$) and healthy controls ($N=73$) in the relation of plasma homocysteine (μmol/L, natural log-transformed) with erythrocyte docosahexaenoic acid (pmol/10e6 erythrocytes).

4. Discussion

The goal of this study was to examine whether relationships between alterations in fatty acid and one-carbon metabolism-allegedly induced by oxidative stress-differed between patients with (recurrent) MDD and matched control subjects. Our main findings were that (I) plasma folate was significantly and similarly positively associated with erythrocyte EPA in both MDD-patients and controls, and (II) homocysteine and erythrocyte DHA were less negatively associated in patients than in controls.

4.1. Folate and EPA

Corresponding with our first hypothesis, plasma folate was significantly positively associated with erythrocyte EPA, DHA and the peroxidation index in patients and controls. However, after correction for confounding factors (marital status, education, socioeconomic status, smoking) only the association of plasma folate with erythrocyte EPA persisted. After additional correction for waist circumference – as a reflection of the metabolic syndrome – this significance persisted at a trend level. Moreover, and contrary to our second hypothesis, patients and controls did not differ in the strength of this association.

To our knowledge, associations between plasma folate and erythrocyte PUFA levels – EPA in particular – have not yet been reported in recurrent MDD-patients. This relationship can be explained by the fact that folate is an essential coenzyme in the methylation processes which modulate enzyme activities of desaturases and elongases required for PUFA synthesis and transport to peripheral tissues including the brain (Selley, 2007; Obeid and Herrmann, 2009; Howard et al., 2014). In addition, folate has been proposed as a direct ROS scavenger and thereby may reflect oxidative stress levels, which may directly affect unsaturated bounds in EPA (Stanger et al., 2009; Fuchs et al., 2001). The finding that the significance of the association was at trend level after additional correction for waist circumference as a reflection of the metabolic syndrome (associated with increased oxidative stress) may support this view.

Of note, interestingly, after correction for confounders, plasma folate was significantly associated with erythrocyte EPA levels only, not with DHA. This could be explained by the different (patho)physiological roles of EPA and DHA, and the differentially regulated effects of oxidative stress of these two omega-3 FAs. EPA is primarily a precursor of eicosanoids, whereas DHA is a/the main determinant of membrane characteristics (Piomelli et al., 2007, McNamara, 2009). This may explain studies suggesting different clinical effects of EPA and DHA (Harris et al., 2008; Davidson, 2013). Therefore, it could be proposed that the regulation of the effects of oxidative stress on EPA and DHA differs. For example, depending on nature, intensity and duration of oxidative stress, the activity of PUFA-desaturases required for synthesis of EPA and DHA may be differentially regulated by DNA methylation with methyl groups derived from folate through the one-carbon-cycle (Lu et al., 2012, Howard et al., 2014). Interestingly, in our previous study of plasma and erythrocyte fatty acid patterns, EPA levels did not differ between depressed cases and controls, while the DHA level was significantly lower in the patients (Assies et al., 2010). Therefore, the results of this study indicate the existence of (I) different mechanism for the influence of oxidative stress on EPA and DHA metabolism, but (II) these mechanisms do not differ between patients and controls.

4.2. Homocysteine and DHA

However, in line with our second hypothesis, homocysteine was less negatively associated with DHA in the patients than in controls. A negative association is consistent with previous studies in healthy, non-depressed subjects, type 2 diabetic subjects and also in schizophrenic patients (Li et al., 2006; Li et al., 2007; Kale et al., 2010; Huang et al., 2012; Huang et al., 2013; Kume et al., 2013). We did not find any association between n-6 PUFAs and parameters of one-carbon metabolism, which was also reported in earlier literature (Kume et al., 2013). Interestingly, in an experimental study, EPA showed a smaller effect than DHA on expression of genes involved in homocysteine metabolism (Huang et al., 2012). Together, these data suggest an independent role for DHA in homocysteine metabolism.

Remarkably, the association between homocysteine and DHA was significantly less negative in patients than in controls. This difference in association between patients and controls persisted after correction for confounding factors. This suggests that patients may be less well able to counteract oxidative stress, which becomes more distinct with increasing (oxidative) stress, whatever its (exogenous) origin (life style, psychological stress) or nature (Mocking et al., 2013). For example due to endogenous enzyme polymorphisms involved in PUFA metabolism, patients may be less flexible in recruiting DHA in response to oxidative stress. Consequently, DHA may decrease less with the rise in homocysteine in patients compared to controls (Lu et al., 2012; Howard et al., 2014).

4.3. Limitations and strengths

Several distinct limitations preventing definite conclusions should be addressed, given that the current study was not originally designed to examine the impact of oxidative stress. First, we only sampled FA and one-carbon metabolites once, and blood was neither drawn in the fasting state nor at a specific time. Second, additional parameters of oxidative stress were not measured. Also structural data on diet, the vitamin, n-3 fatty acid and other supplementations were not available at sampling time. In addition, our study was not set up to specifically test the effects of other medications or specific somatic comorbidity. Consequently, the present study does not have enough power to detect these possible influences. These potential confounding factors may have influenced our results, and should be taken into account in future studies. Finally, we did not differentiate according to lipid subclass, e.g. phosphatidylcholine, sphingolipids, lipid peroxidation products.

Nevertheless, our study also had particular strengths. First, to our knowledge, this is the largest sample in which the one-carbon and fatty acid metabolism were studied in combination. This provides more power to detect differences and similarities in the associations between these systems. Second, we were able to include a specific sample of highly recurrent MDD patients and carefully matched controls. This patient population is thought to be particularly prone to the detrimental effects of oxidative stress on CVD-development, and thereby provides an optimal means to study oxidative stress induced effects on the associations between one-carbon and FA-metabolism.

4.4. Suggestions for further research

Particularly prospective studies in large samples, as well as RCTs, should include not only relevant parameters of FA-metabolism and one-carbon metabolism, but also clinically relevant parameters of (oxidative) stress (Giustarini et al., 2009). Moreover, reliable measurements of (non-)enzymatic lipid peroxidation products should be included. It is increasingly shown that (non-) enzymatic oxidation products of PUFAs are more sensitive indicators of the clinical relevance and impact of oxidative stress and the cell danger response than their precursors/parent PUFAs (Naviaux, 2012; Naviaux, 2014; Delmastro-Greenwood et al., 2014).

4.5. Implications for the clinic

The pathophysiology of the co-occurrence of MDD and CVD still has to be further elucidated. Evidence for a causal role of oxidative stress is rapidly increasing (Giustarini et al., 2009). Combining and defining clinical and biochemical criteria in the clinic may help in identifying patients that might benefit from timely overcoming/correction of oxidative stress (e.g. by means of lifestyle interventions) to prevent and treat (CVD in) psychiatric disorders (Assies et al., 2014). More specific, seen as potential indicators of a more biologically characterized MDD-subtype, findings in (recurrent) MDD-patients may be specifically linked to recurrence and CVD-risk.

In conclusion, we compared the strength of associations between parameters of two systems involved in oxidative stress regulation (FA- and one-carbon metabolism) in patients with (recurrent) MDD and matched control subjects. We found that (I) plasma folate was significantly and similarly positively associated with EPA in patients and controls suggesting a similar handling of oxidative stress up to a certain level, but (II) DHA and homocysteine were less negatively associated in patients than in controls. The latter may indicate an independent role of DHA in the metabolism of homocysteine levels and handling of oxidative stress, which may differ in patients with recurrent MDD compared to controls depending on the nature, intensity and duration of oxidative stress.

Acknowledgments

All authors report no potential conflicts of interest.

Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2015.06.025.

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