Polyunsaturated fatty acid associations with dopaminergic indices in major depressive disorder

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Abstract

Dopaminergic function is thought to be altered in major depression and, in animal studies, is reduced in omega-3 polyunsaturated fatty acid (PUFA) deficiency states. Therefore we studied PUFAs and resting prolactin, a marker for dopaminergic tone, and cerebrospinal fluid homovanillic acid (HVA), the chief dopamine metabolite. In medication-free adults (n=23) with DSM-IV major depressive disorder (MDD), we measured plasma phospholipid levels of omega-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), the omega-6 PUFA arachidonic acid (AA), and plasma prolactin levels before and after administration of dl-fenfluramine (FEN). In a subset of patients (n=14), cerebrospinal fluid levels of HVA and the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were obtained through lumbar puncture. Baseline prolactin was negatively correlated with omega-3 PUFAs (logDHA, F1,21=20.380, p<0.001; logEPA, F1,21=10.051, p=0.005) and positively correlated with logAA:DHA (F1,21=15.263, p=0.001), a measure of omega-6/omega-3 balance. LogDHA was negatively correlated with CSF HVA (Spearman’s ρ =−0.675, p=0.008) but not 5-HIAA (Spearman’s ρ =−0.143, p=0.626) after controlling for sex and HVA − 5-HIAA correlation. PUFAs did not predict the magnitude of the FEN-stimulated change in prolactin, considered to be a serotonin effect. The robust relationship of omega-3 PUFAs with dopaminergic but not serotoninergic indices suggests that omega-6:omega-3 balance may impact depression pathophysiology through effects on the dopaminergic system.

Key words: Dopamine, major depressive disorder, omega-3, polyunsaturated fatty acids, prolactin.

Introduction

Long-chain polyunsaturated fatty acids (PUFAs) are dietary nutrients essential for normal brain functioning. Low peripheral levels of omega-3 PUFA and higher omega-6:omega-3 ratios are associated with major depression (Edwards et al., 1998; Peet et al., 1998; Maes et al., 1999; Mamalakis, 2002; De Vriese, 2003; Lin et al., 2010). Although publication bias is a limitation of the clinical trial literature concerning omega-3 PUFA supplements in depression (Bloch and Hannonstad, 2012b), most meta-analyses find supplements to be effective when two conditions are met: subjects have major depressive episodes (Martins, 2009; Appleton et al., 2010), and supplements contain at least 60% eicosapentaenoic acid (EPA) (Martins, 2009; Sublette et al., 2011; Lin et al., 2012; Martins et al., 2012). With these higher EPA concentrations, standard mean differences compared to placebo are similar to those in pharmaceutical antidepressant trials (Martins et al., 2012). Therefore, understanding how PUFAs may influence the pathophysiology of major depression has important clinical implications.

Animal studies identify many effects of PUFAs in the dopamine system. Dietary deficiency of omega-3 PUFAs lowers levels of dopamine (de la Presa Owens and Innis, 1999), D2 receptors, D2 receptor mRNA and dopaminergic presynaptic vesicles (Zimmer et al., 2000a), and increases breakdown of dopamine (Zimmer et al., 1998), in the prefrontal cortex. Omega-3 PUFA deficiency also results in decreased tyrosine hydroxylase (Kuperstein et al., 2008), the rate-limiting enzyme in dopamine synthesis and the main target of prolactin feedback regulation of dopamine (Arbogast and Voogt, 1991), and fewer detectable dopaminergic neurons in the substantia nigra and ventral tegmentum (Ahmad et al., 2008), but higher dopamine levels, D2 receptor mRNA,
Maternal omega-3 PUFA deficiency results in elevated post-natal expression of dopamine receptor genes in rat pups (Kuperstein et al., 2005). Dietary supplementation with omega-3 fatty acids increases dopamine levels and D2 receptor binding, and lowers monoamine oxidase B (MAO-B) activity in the prefrontal cortex and D2 receptor binding in the striatum (Chalon et al., 1998).

Abnormalities of dopamine function are implicated in major depression (see Willner (1983a,b,c) and Kapur (reviewed in (Dunlop and Nemeroff, 2007)). However, very little is known about relationships between PUFA status and dopaminergic functioning in major depression.

We examined associations between plasma phospholipid PUFAs and dopamine functioning in medication-free participants with major depressive disorder (MDD) during a fenfluramine (FEN) challenge paradigm, using two approaches. (1) Correlations were tested between PUFAs and baseline or FEN-stimulated plasma levels of prolactin, as surrogates for dopaminergic and serotonergic function, respectively. Prolactin and dopamine have a negative feedback relationship, such that low levels of prolactin serve as an indicator of high dopamine. (2) To assess rates of dopamine metabolism, we studied correlations between plasma levels of PUFAs and cerebrospinal fluid (CSF) levels of homovanillic acid (HVA), the principal dopamine metabolite. To compare with effects on serotonergic functioning, we also assessed effects of PUFAs on CSF levels of the serotonergic metabolite, 5-hydroxyindoleacetic acid (5-HIAA). We hypothesized that low levels of omega-3 PUFAs, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), and high ratios of the omega-6 PUFA, arachidonic acid (AA, 20:4n-6), to DHA (AA:DHA) would be associated with lower dopamine activity as reflected in higher prolactin and lower CSF HVA.

**Method**

**Sample**

Participants (n=23) were adults, aged 20–72 yr, who provided written informed consent to participate in IRB-approved mood disorders studies at the New York State Psychiatric Institute (NYSPI). All participants had DSM-IV (SCID I) (First et al., 1997) diagnoses of a current Major Depressive Episode in the context of MDD. Psychiatric exclusions were any other current Axis I disorders except anxiety disorders, and substance abuse within 2 months previously or substance dependence within 6 months. Axis II disorders were permitted. Participants did not have active medical or neurologic illness based on history, physical examination, and a battery of standard laboratory tests. They were not on psychotropic medications for at least 2 wk prior to the collection of blood samples (6 wk for fluoxetine, 4 wk for antipsychotic medications) with the exception of lorazepam, which was permitted for insomnia or anxiety in doses of up to 3 mg daily until 3 d before scanning. The clinician-administered 17-item Hamilton Depression Rating Scale (HDRS-17) (Hamilton, 1960) and the participant self-report Beck Depression Inventory (BDI) (Beck et al., 1996) were used to assess depression severity. Lifetime aggression was estimated with the Brown–Goodwin Aggression History Scale (Brown et al., 1979). Data from these research participants have been used in previous studies with different research objectives, with respect to PUFAs (Sublette et al., 2006, 2009), prolactin (Malone et al., 1996; Oquendo et al., 2003; Sher, 2003; Keilp et al., 2010) and CSF HVA (Sher et al., 2006).

**Fenfluramine administration and plasma prolactin determination**

Two blood samples were taken prior to FEN administration and again at hourly intervals up to 5 h afterward as described in detail elsewhere (Mann et al., 1992). Patients were administered one dose of approximately 0.8 mg/kg of oral FEN. Prolactin levels were assayed by immunoradiography kit (Hybritech Inc, USA).

**Cerebrospinal fluid homovanillic acid and 5-hydroxyindoleacetic acid determination**

Acquisition of CSF and determination of HVA and 5-HIAA levels were performed as previously described (Placidi et al., 2001) in a subset of participants (N=14). Briefly, fasting participants had lumbar puncture at about 8:00 hours after bed rest from midnight. After an initial 1 ml was removed,15 ml of CSF were collected on ice and centrifuged under refrigeration. The supernatant was stored at −70 °C in 1 ml aliquots and assayed using high-performance liquid chromatography (Scheinin et al., 1983).

**Plasma PUFA determination**

Fasting morning blood samples were collected and plasma PUFAs were shipped on dry ice for analysis at the National Institute of Mental Health using modified standard procedures (Folch et al., 1957; Morrison and Smith, 1964; Kim and Salem, 1990), as described in detail previously (Sublette et al., 2009). Briefly, total plasma lipids were extracted (Folch et al., 1957), from which total plasma phospholipids were separated using solid-phase extraction (Kim and Salem, 1990), methylated (Morrison and Smith, 1964), and separated by gas chromatography with flame ionization. Peaks were identified using standards (NuChek Prep, USA) and quantified using a 23:0 internal standard.
**Statistical analyses**

Statistical analyses were performed using IBM-SPSS-Statistics (v.20 for Mac (Apple, Inc., USA)). Log-transformations were used for EPA (logEPA, DHA (logDHA), the ratio of logAA to logDHA (logAA:DHA) levels, the analogous PUFA as a percentage of total plasma phospholipid PUFA levels (logPUFA%, logDHA %, logEPA%, logAA%:DHA%), and stimulated prolactin levels, due to leftward skewed distributions. The baseline prolactin level was defined as the mean of the two pre-FEN levels. The stimulated prolactin level was estimated as described previously (Keilp et al., 2010) as the net maximal difference between the baseline and post-FEN prolactin levels. Post-hoc analyses used total area under the curve (Pruessner et al., 2003) as another serotonin stimulated prolactin estimate. Separate linear regression analyses were performed with baseline and stimulated prolactin levels as the dependent variables and logPUFA levels (logDHA, logEPA, and logAA:DHA) as predictors. Similarly, to estimate relationships between CSF HVA or 5-HIAA and logPUFA, linear regression analyses were performed with CSF HVA or 5-HIAA levels as the dependent variable and logPUFA levels (logDHA, logEPA, and logAA:DHA) as predictors, including as covariates those characteristics found to be correlated with PUFA. Additional exploratory analyses repeated the regression analyses using logDHA%, logEPA% and logAA%:DHA%, since absolute and relative PUFA levels can have different functional significance. We estimated omega-3 PUFA effects on dopamine compared to effects on serotonin by examining the relationships between logPUFA and HVA or 5-HIAA, respectively. Since HVA and 5-HIAA are correlated ($r = 0.71, p=0.005$), we performed partial correlation studies using Spearman’s coefficient, of logPUFA correlating with HVA controlling for 5-HIAA, and the opposite, logPUFA correlating with 5-HIAA controlling for HVA. For all analyses, $p \leq 0.05$ was considered significant. No corrections were made for multiple testing.

**Results**

**Sample characteristics**

Participants’ demographic and clinical characteristics are shown in Table 1. When age, sex, HDRS-17 scores, total aggression, attempter status, smoking status and body-mass index were tested for correlations with logPUFA, only sex correlated with logDHA ($r = -0.449, p=0.031$) and logAA:DHA ($r=0.433, p=0.039$), but not logEPA ($r=-0.320, p=0.137$) and for consistency was used as a covariate in all subsequent analyses. Mean plasma levels of logDHA were lower in females (3.40 ± 0.45) than in males (3.79 ± 0.32). The mean number of total depressive episodes was 3.74 ± 2.1 and the mean length of the current episode was 36.4 ± 68.1 weeks. There were 13 participants with a prior history of substance use disorder, three of whom had disorders involving multiple substances. Seven of the participants had current comorbid anxiety disorders, and three of them had more than one anxiety diagnosis. Additionally, 16 participants had an Axis II comorbidity, seven of which were borderline personality disorders.

**Relationship of plasma PUFA levels to baseline prolactin levels**

Baseline prolactin negatively correlated with plasma phospholipid levels of omega-3 PUFAs and positively correlated with omega-6 PUFA logAA:DHA levels (see Fig. 1, Table 2). Levels of log PUFA% likewise correlated with baseline prolactin, although less robustly (data not shown).

**Relationship of plasma phospholipid PUFA levels to fenfluramine-stimulated prolactin levels**

Peak FEN-stimulated prolactin levels were negatively correlated only with logDHA (see Table 2). The magnitude of the change in prolactin was not correlated with logPUFA levels, neither as the maximal difference between peak and baseline prolactin (data not shown), nor as total area under the curve (data not shown).

**Relationship of plasma phospholipid PUFA levels to CSF homovanillic acid or 5-hydroxyindoleacetic acid levels**

CSF HVA negatively correlated with logDHA (see Table 2, Fig. 1d), but not with logEPA or logAA:DHA (data not shown). The DHA–HVA correlation remained significant after controlling for 5-HIAA (Spearman’s $r = −0.675, p=0.008$). Whereas, after controlling for HVA,

**Table 1. Demographic and clinical characteristics of participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
<th>Mean (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: male</td>
<td>11 (48%)</td>
<td></td>
</tr>
<tr>
<td>Race: white</td>
<td>19 (83%)</td>
<td></td>
</tr>
<tr>
<td>Suicide attempter</td>
<td>11 (48%)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>7 (30%)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>41.3 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>27.3 (5.2)</td>
<td></td>
</tr>
<tr>
<td>Hamilton depression rating scale (17-item)</td>
<td>21.9 (4.4)</td>
<td></td>
</tr>
<tr>
<td>Beck depression inventory</td>
<td>33.2 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Brown-goodwin aggression history scale</td>
<td>17.7 (6.5)</td>
<td></td>
</tr>
<tr>
<td>DHA (µg/ml)</td>
<td>39.2 (15.5)</td>
<td></td>
</tr>
<tr>
<td>EPA (µg/ml)</td>
<td>7.2 (4.3)</td>
<td></td>
</tr>
<tr>
<td>AA (µg/ml)</td>
<td>113.9 (30.7)</td>
<td></td>
</tr>
<tr>
<td>Baseline prolactin (ng/ml)</td>
<td>8.1 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Peak prolactin (ng/ml)</td>
<td>17.0 (8.1)</td>
<td></td>
</tr>
<tr>
<td>HVA (pmol/ml)</td>
<td>215.7 (114.1)</td>
<td></td>
</tr>
<tr>
<td>5-HIAA (pmol/ml)</td>
<td>90 (33.8)</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Values are presented as mean (standard deviation).*

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**Statistical analyses**

Statistical analyses were performed using IBM-SPSS-Statistics (v.20 for Mac (Apple, Inc., USA)). Log-transformations were used for EPA (logEPA), DHA (logDHA), the ratio of logAA to logDHA (logAA:DHA) levels, the analogous PUFA as a percentage of total plasma phospholipid PUFA levels (logPUFA%, logDHA %, logEPA%, logAA%:DHA%), and stimulated prolactin levels, due to leftward skewed distributions. The baseline prolactin level was defined as the mean of the two pre-FEN levels. The stimulated prolactin level was estimated as described previously (Keilp et al., 2010) as the net maximal difference between the baseline and post-FEN prolactin levels. Post-hoc analyses used total area under the curve (Pruessner et al., 2003) as another serotonin stimulated prolactin estimate. Separate linear regression analyses were performed with baseline and stimulated prolactin levels as the dependent variables and logPUFA levels (logDHA, logEPA, and logAA:DHA) as predictors. Similarly, to estimate relationships between CSF HVA or 5-HIAA and logPUFA, linear regression analyses were performed with CSF HVA or 5-HIAA levels as the dependent variable and logPUFA levels (logDHA, logEPA, and logAA:DHA) as predictors, including as covariates those characteristics found to be correlated with PUFA. Additional exploratory analyses repeated the regression analyses using logDHA%, logEPA% and logAA%:DHA%, since absolute and relative PUFA levels can have different functional significance. We estimated omega-3 PUFA effects on dopamine compared to effects on serotonin by examining the relationships between logPUFA and HVA or 5-HIAA, respectively. Since HVA and 5-HIAA are correlated ($r = 0.71, p=0.005$), we performed partial correlation studies using Spearman’s coefficient, of logPUFA correlating with HVA controlling for 5-HIAA, and the opposite, logPUFA correlating with 5-HIAA controlling for HVA. For all analyses, $p \leq 0.05$ was considered significant. No corrections were made for multiple testing.

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there was no significant relationship between DHA and 5-HIAA (Spearman’s \( \rho = -0.143, p = 0.626 \)). No effects were observed for any logPUFA% on HVA (data not shown).

**Discussion**

Dopamine is a key regulator of prolactin (Fitzgerald and Dinan, 2008) which, based on a short loop feedback relationship (Ben-Jonathan and Hnasko, 2001), serves in turn as an indirect, inverse indicator of dopaminergic tone. Thus our observations that plasma omega-3 PUFAs inversely correlated with baseline prolactin may mean, but do not prove, a concomitant positive correlation with dopamine in MDD. Sex effects on DHA are consistent with both animal studies, in which female rats showed differential rates of DHA synthesis (Alessandri et al., 2012) and a greater effect of DHA on synaptic function (Perez et al., 2010), and epidemiologic studies in which associations between depressive symptoms and infrequent fish consumption were much more pronounced in women than men (Tanskanen et al., 2001; Timonen et al., 2004; Colangelo et al., 2009).

Since in mammals omega-3 PUFAs DHA and EPA cannot be synthesized de novo, their plasma levels reflect primarily dietary intake and a small amount of transformation from the precursor a-linolenic acid (ALA, 18:3n-3) (Bezard et al., 1994; Goyens et al., 2005). Thus, when considered together with animal studies of omega-3 deficiency effects on dopamine status (de la Presa Owens and Innis, 1999; Zimmer et al., 1998,
Our findings suggest that dietary intake of omega-3 PUFAs, both absolute and relative to omega-6 status, may contribute to regulation of brain dopaminergic functioning in humans. We have focused on omega-3 PUFA since meta-analysis of depression studies finds that DHA, EPA and total omega-3 PUFAs are low in depression, while neither AA nor total omega-6 PUFA levels differ (Balcioglu and Wurtman, 1998). However, this may be primarily because DHA or EPA concentrations are much smaller in magnitude than AA, and thus the ratio is most sensitive to changes in the denominator. A lack of AA relevance for dopaminergic signaling is also suggested by findings that both fluoxetine (Lee et al., 2007) and imipramine (Lee et al., 2010), which primarily inhibit serotonin and norepinephrine (in the case of imipramine) reuptake, increase AA-specific phospholipase A2 activation and AA turnover in phospholipids; whereas bupropion (Lee et al., 2010), which primarily inhibits dopamine reuptake, has no effects on AA turnover. The involvement of AA may be a pathophysiologic feature of bipolar disorder in contradistinction to major depressive disorder, as multiple medications with mood stabilizing properties have been found to affect the AA cascade (Rao and Rapoport, 2009).

The observed correlation of DHA with CSF HVA but not with CSF 5-HIAA indicates that dietary PUFA intake may be specifically associated with dopaminergic rather than serotonergic functioning. This hypothesis is also supported by our finding that PUFAs levels did not correlate with the magnitude of FEN-stimulated change in prolactin levels, since FEN primarily stimulates serotonin release without significant effects on dopamine (Balcicoglu and Wurtman, 1998).

Our observation that DHA correlated negatively with CSF HVA indicates that there is a significant connection between omega-3 status and dopaminergic tone in the brain. The directionality of the correlation, however, was contrary to our expectations, and indicates that the relationship between omega-3 PUFA and dopaminergic functioning is likely complex. This complexity is also seen in the only other published human studies of PUFA relationships to CSF HVA and 5-HIAA in clinical populations of which we are aware, in which negative correlations were also reported between DHA and CSF 5-HIAA and CSF HVA in early-onset alcoholics (Hibbeln et al., 1998a) and in participants with a history of violence (Hibbeln et al., 1998b) (at a trend level for HVA), whereas positive associations with both HVA and 5-HIAA were seen in healthy volunteer and late-onset alcoholic groups (Hibbeln et al., 1998a,b). However, those analyses did not control for effects of the correlation between HVA and 5-HIAA.

Additionally, our CSF findings may relate to preclinical observations that chronic omega-3 PUFA deficiency causes increased levels of both dopamine metabolites HVA and 3,4-dihydroxyphenylacetic acid (DOPAC) in the prefrontal cortex of rats (Zimmer et al., 1998).

Table 2. Regression models of logPUFA predicting plasma baseline and peak prolactin and CSF homovanillic acid levels, controlling for sex

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictors</th>
<th>β</th>
<th>95% CI</th>
<th>t (df=20)</th>
<th>p</th>
</tr>
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<tr>
<td>Baseline plasma prolactin</td>
<td>logDHA</td>
<td>−3.944</td>
<td>−6.343 −3.429</td>
<td>−3.429</td>
<td>0.003</td>
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<td></td>
<td>Sex</td>
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<td>−3.849 −1.841</td>
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<tr>
<td></td>
<td>logEPA</td>
<td>−2.150</td>
<td>−3.902 −2.558</td>
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<tr>
<td></td>
<td>Sex</td>
<td>−2.487</td>
<td>−4.596 −2.461</td>
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<td>0.023</td>
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<tr>
<td></td>
<td>logAA:DHA</td>
<td>4.207</td>
<td>0.408 2.310</td>
<td>2.310</td>
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<tr>
<td></td>
<td>Sex</td>
<td>−1.446</td>
<td>−4.097 −1.138</td>
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<tr>
<td>Peak plasma prolactin</td>
<td>logDHA</td>
<td>−7.567</td>
<td>−13.454 −1.679</td>
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<td></td>
<td>Sex</td>
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<td>−13.431 −3.398</td>
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<td></td>
<td>logEPA</td>
<td>−3.512</td>
<td>−7.794 0.771</td>
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<td></td>
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<td></td>
<td>logAA:DHA</td>
<td>5.954</td>
<td>−3.348 15.256</td>
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<td></td>
<td>Sex</td>
<td>−8.668</td>
<td>−15.159 −2.177</td>
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<td>CSF homovanillic acid</td>
<td>logDHA</td>
<td>−255.476</td>
<td>−415.715 −117.238</td>
<td>−117.238</td>
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<tr>
<td></td>
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<td></td>
<td>logEPA</td>
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<td>−294.278 82.446</td>
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<td>−200.854 420.974</td>
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<td></td>
<td>Sex</td>
<td>90.289</td>
<td>−130.463 311.042</td>
<td>311.042</td>
<td>0.387</td>
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</table>
Our findings are consistent with a model in which low omega-3 PUFA levels are associated with a constitutively greater rate of dopamine breakdown, resulting in high HVA, low dopamine, and high prolactin due to less dopaminergic inhibition. However, this model is hypothetical, since our research design does not provide information about causality or directionality of effects, and since HVA was measured in CSF while prolactin was measured in plasma. Future studies could test for effects of omega-3 PUFA deficiency on catechol-O-methyl transferase (COMT) or MAO, enzymes which control dopamine breakdown.

Lower dopaminergic neurotransmission has been proposed as a pathophysiological mechanism in a subgroup of depressed patients, based both on the known beneficial effects of dopamine on cognitive functioning and reward circuitry, and on the antidepressant effectiveness of medications such as monoamine oxidase inhibitors, bupropion, and pramipexole, that enhance dopamine neurotransmission (Dunlop and Nemeroff, 2007). In support of this hypothesis, a number of animal studies (Carboni et al., 1990; Tanda et al., 1997; Devoto et al., 2004; Masana et al., 2011, 2012; Hudson et al., 2012) find that noradrenergic antidepressant treatments facilitate dopamine transmission. If true, this mechanistic explanation could account for the failure of SSRIs to work in some depressed patients due to a lack of influence on the dopaminergic system.

The hypothesis that omega-3 intake may have therapeutic effects through dopamine neurotransmission in major depression is consistent with findings in randomized placebo-controlled trials that antidepressant efficacy of omega-3 supplementation is equivalent to and also additive to that of the serotonergic reuptake inhibitor fluoxetine (Jazayeri et al., 2008); and similarly additive to the effects of citalopram (Gertsik et al., 2012). Our findings also raise the possibility that omega-3 supplementation may have specific value for MDD with a dopaminergic system deficit. However, we cannot rule out the possibility of opposite directionality, i.e. that dopaminergic tone could affect PUFA levels by influencing turnover or metabolism or, on an organismic level, by affecting appetite and/or food choices.

We found stronger relationships between PUFA status and prolactin with respect to DHA compared to EPA, and an association of DHA to CSF HVA but not to 5-HLAA levels. The relative importance of DHA and EPA levels in depression pathophysiology and in treatment seems to be different. In a meta-analysis of 14 studies in peer-reviewed journals that compared depressed patients with healthy volunteers with respect to PUFA levels in red blood cell membranes, blood phospholipids or cholesteryl esters, group differences were found with regard to both DHA and EPA (Lin et al., 2010). In clinical trials, however, meta-analyses by us (Sublette et al., 2011) and others (Ross et al., 2007; Martins, 2009; Lin et al., 2012; Martins et al., 2012) have found that higher proportions of EPA in the omega-3 supplements are crucial for efficacy, although negative publication bias may diminish enthusiasm (Bloch and Hannestad, 2012a,b) for this finding, which is also counter-intuitive given that DHA is vastly more abundant in the brain than EPA. Our findings implicate DHA more strongly than EPA in dopamine-related MDD pathophysiology.

Potential mechanisms whereby omega-3 PUFA levels may regulate dopamine include reducing cortical MAO-B activity, thereby decreasing the degradation of monoamines (Chalon et al., 1998); improving dopamine neuron survival (Ahmad et al., 2008); and influencing lipid rafts (Rockett et al., 2011, 2012; Williams et al., 2012; Larson et al., 2013), which, in turn, may upregulate D1 receptors (Yu et al., 2004) and mediate D2 receptor endocytosis (Genedani et al., 2005).

**Study limitations**

Future studies in larger cohorts are required to replicate these findings. PUFA data were only available for depressed participants; thus, these results provide no information about PUFA-prolactin relationships in healthy individuals for comparison with MDD. Moreover, in this depressed sample, there is no way to distinguish between a trait and state effect; nor, in this small sample, can we parse out whether the PUFA-prolactin relationships are more relevant for particular depression subtypes. We also note that race in this particular sample is disproportionately white, so the relevance for other racial groups is not clear. We chose to study the plasma phospholipid fraction, which is relatively enriched in EPA and DHA, over total plasma PUFA levels, in which percentages of n-3 PUFA may be more strongly influenced by the relative amounts of different lipoproteins that exhibit distinct PUFA compositions (Hodson et al., 2008), erythrocytes, in which quantitation is not absolute, or unesterified PUFAs, which are the most difficult to quantify due to their very small proportion. However, PUFA indices other than plasma phospholipids may yield different results. The extent to which peripheral PUFA measures reflect human brain function is unknown, although we have reported brain-region-specific correlations between plasma phospholipid PUFA levels and relative regional cerebral glucose utilization as measured by uptake of [18F]-fluoro-2-deoxyglucose on positron emission tomography (PET) scanning in a sample that included these MDD subjects plus six subjects with bipolar disorder (Sublette et al., 2009).

**Conclusions**

Omega-3 PUFA deficiencies may impact major depression in part through effects on the dopaminergic system. This finding may have important implications for therapeutic strategies involving augmentation of standard antidepressant medications with fish oil.
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