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Brief report

Selective deficits in erythrocyte docosahexaenoic acid composition in adult patients with bipolar disorder and major depressive disorder

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ABSTRACT

Background: Epidemiological and controlled intervention trials suggest that omega-3 (n-3) fatty acid deficiency represents a reversible risk factor for recurrent affective disorders. However, there is limited comparative information available regarding the n-3 fatty acid status and associated mood symptoms in medication-free patients with major depressive disorder (MDD) and bipolar disorder (BD).

Methods: The fatty acid composition of erythrocyte membranes from adult male and female healthy controls ($n=20$) and medication-free patients with MDD ($n=20$) and BD ($n=20$) was determined by gas chromatography. Associations with depression and mania symptom severity scores were investigated.

Results: After correction for multiple comparisons, both MDD (−20%) and BD (−32%) patients exhibited significantly lower erythrocyte docosahexaenoic acid (DHA, 22:6n-3) composition relative to healthy controls, and there was a trend for lower DHA in BD patients relative to MDD patients (−15%, $p=0.09$). There were no gender differences for DHA in any group. Other n-3 fatty acids, including eicosapentaenoic acid (EPA, 20:5n-3) and docosapentanoic acid (22:5n-3), and n-6 fatty acids, including arachidonic acid (AA, 20:4n-6), were not different. Erythrocyte DHA composition was inversely correlated with indices of delta-9 desaturase activity (18:1/18:0), and associated elevations in oleic acid (18:1n-9) composition, and delta-6 desaturase activity (20:3/18:2). DHA composition was not significantly correlated with depression or mania symptom severity scores.

Limitations: Data regarding diet and life style factors (cigarette smoking) were not available to evaluate their contribution to the present findings.

Conclusions: Male and female patients with MDD and BD exhibit selective erythrocyte DHA deficits relative to healthy controls, and this deficit was numerically greater in BD patients. Selective DHA deficits are consistent with impaired peroxisome function, which has implications for n-3 fatty acid interventions aimed at preventing or reversing this deficit.

1. Introduction

Evidence from cross-national and cross-sectional epidemiological surveys suggest that greater habitual dietary omega-3 (*n*-3) fatty acid intake from fish/seafood, principally eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), is associated with reduced prevalence rates for major depressive disorder (MDD), particularly among women (Hibbeln, 1998, 2002; Peet, 2004; Raeder et al., 2007; Tanskanen et al., 2001; Timonen et al., 2004), and bipolar disorders (BD) (Noaghiul and Hibbeln, 2003).

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Prospective longitudinal studies have found that baseline n-3 fatty acid intake is associated with reduced adjusted risk for emergent depressive symptoms (Golding et al., 2009; Kamphuis et al., 2006; Rees et al., 2009). In one of the largest prospective studies conducted to date, greater EPA and DHA intake from fish at baseline was associated with a lower adjusted risk for depression at 10 years among 3317 young adults (mean age: 32 years) residing in the U.S., and this inverse association was stronger in women (Colangelo et al., 2009). Additionally, independent meta-analyses have also found a significant advantage of EPA + DHA supplementation over placebo for reducing depression symptom severity (Freeman et al., 2006; Lin and Su, 2007). These data suggest that n-3 fatty acid deficiency may represent a preventable and reversible risk factor for recurrent mood disorders.

Because erythrocyte EPA + DHA composition is positively correlated with habitual dietary EPA + DHA intake (Cao et al., 2006; Sands et al., 2005; Itomura et al., 2008), it represents a peripheral biomarker of n-3 fatty acid status. Prior case–control studies have observed significant erythrocyte or plasma EPA and/or DHA deficits in patients with MDD (Maes et al., 1996, 1999; Peet et al., 1998; Edwards et al., 1998; Tiemeier et al., 2003) and BD (Chiu et al., 2003; Ranjekar et al., 2003) relative to healthy subjects residing in western European and Asian countries. Only one case–control study has been conducted in the U.S., and found no significant difference in plasma free or esterified DHA or EPA in a small sample ($n=10$) of medication-free manic patients (Sublette et al., 2009). Some studies have observed an inverse correlation between DHA and/or EPA, and positive correlations between plasma n-6:n-3 ratios, principally AA:EPA, and measures of depression and/or manic symptom severity (Adams et al., 1996; Edwards et al., 1998; Conklin et al., 2007; Maes et al., 1996; Sublette et al., 2009). However, there have been no studies directly comparing MDD and BD patients to identify potential distinguishing features in erythrocyte fatty acid composition and associated mood symptoms to evaluate the diagnostic utility of this biomarker.

In the present study, we determined erythrocyte fatty acid composition in healthy adult male and female controls without a history of psychiatric illness ($n=20$) and medication-free patients with MDD ($n=20$) or BD ($n=20$). We used medication-free patients because preclinical evidence suggests that antidepressant and mood-stabilizer medications alter membrane fatty acid turnover and/or biosynthesis (Qu et al., 2006; Raeder et al., 2006), and may confound associations between fatty acid status and symptom severity scores. We additionally evaluated the effects of gender because gonadal hormones have a significant influence on n-3 fatty acid biosynthesis and erythrocyte composition (Bakewell et al., 2006; Giltay et al., 2004; McNamara et al., 2009; Pawlosky et al., 2003), and we have observed gender differences in postmortem cortical DHA deficits in patients with MDD (female > male)(McNamara et al., 2007). Additionally, we evaluated the relationship between erythrocyte fatty acid compositions and depression and manic symptom severity scores on the Hamilton Depression Rating Scale (HDRS, Hamilton, 1967) and the Clinician-Administered Rating Scale for Mania (CARS-M, Altman et al., 1994), respectively. Based on the evidence reviewed above, our primary hypothesis was that erythrocyte DHA composition would be significantly lower in MDD and BD patients relative to age-matched healthy controls, and inversely correlated with depression symptom severity scores.

2. Method

2.1. Subject demographics

These studies were conducted in hospitalized patients with BD ($n=20$) and MDD ($n=20$) admitted to the Psychiatric Clinical Research Center, as part of the General Clinical Research Center (GCRC), University of Illinois at Chicago. This study was approved by the Institutional Review Board of the University of Illinois at Chicago. Healthy adult male and female controls with no history of psychiatric illness ($n=20$) were recruited from the greater Chicago area. Patients were kept drug-free up to 2 weeks prior to blood collection to permit sufficient washout for the majority of antidepressant and mood-stabilizer medications. Although some patients were receiving fluoxetine, and norfluoxetine has a long elimination half-life (7–15 days, Altamura et al., 1994), we have previously found that chronic treatment with fluoxetine resulting in clinically-relevant plasma fluoxetine and norfluoxetine concentrations did not alter rat erythrocyte fatty acid composition (Able et al., 2009). A comparison of group demographic variables is presented in Table 1. Data regarding smoking status and diet were not obtained. There were no significant group differences in age, $F(2,54)=1.3$, $p=0.28$. Mean depression (HDRS) and manic (CARS-M) symptom severity scores are presented.

2.2. Erythrocyte fatty acid composition

Whole venous blood (40 ml) was collected into tubes containing 4 ml of sodium citrate (3.8%), and centrifuged at $210\times g$ for 15 min at 4 °C. Plasma and the platelet rich interface were removed, and erythrocytes washed twice with 0.9% saline and stored at −80 °C. Erythrocyte membrane total fatty acid composition was determined with a Shimadzu GC-2014 (Shimadzu Scientific Instruments Inc., Columbia MD) using the saponification and methylation procedure described previously (Metcalfe et al., 1966). Analysis of fatty acid methyl esters was based on the area under the curve calculated with Shimadzu Class VP 4.3 software. Fatty acid identification was based on retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap PA). Data are expressed as weight percent of total fatty acids (mg fatty acid/100 mg fatty acids). To avoid potential variability associated with the manual addition of an internal

Table 1
Subject demographics.

Variable	HC	MDD	BD
Age (years), mean ± SD	36.2 ± 8.9	34.6 ± 11.0	30.7 ± 11.7
Gender	10M, 10F	6M, 11F	12M, 6F
Race	3AS, 7AA, 1H, 9C	8AA, 9C	6AA, 12C
HDRS	–	26.4 ± 7.9	17.6 ± 13.3
CARS-M	–	5.0 ± 4.7	15.0 ± 12.3

AS, Asian; AA, African American; H, Hispanic; C, Caucasian.

standard, we did not calculate total mass (nmol/g). However, we have previously found that weight percent data and total mass (nmol/g) data are highly correlated using the direct saponification method (McNamara et al., 2008a). All samples were processed by a technician blinded to group identity.

2.3. Statistical analysis

Analyses of variance (ANOVA) were performed using GB-STAT (V.10, Dynamic Microsystems, Inc., Silver Springs MD). Pending a significant main effect of group (HC, MDD and BD) after Bonferroni correction for multiple comparisons ($\alpha = 0.05/17$ fatty acids and ratios $= 0.003$), Bonferroni corrected post-hoc tests (2-tailed) were performed to evaluate individual group differences. Gender × group interactions were evaluated with a two-factor ANOVA using Illness (HC, MDD and BD) and Gender (male and female) as the main factors. Parametric linear regression analyses were performed to determine the interrelationship between erythrocyte fatty acid composition and relevant fatty acids ratios and depression (HDRS) and mania (CARS-M) symptom severity scores, and Bonferroni-adjusted for multiple comparisons ($\alpha = 0.05/16$ comparisons $= 0.003$).

3. Results

3.1. Fatty acid composition

Erythrocyte samples from three MDD patients and two BD patients were excluded from the final analyses because of evidence for gross abnormalities in membrane fatty acid composition, including the absence of DHA. Therefore, the analyses were performed on $n = 20$ healthy controls, $n = 17$ MDD patients, and $n = 18$ BD patients. After correction for multiple comparisons, significant main effects of group were observed for DHA (22:6*n*-3), $F(2,54) = 11.6$, $p \leq 0.0001$, oleic acid (18:1*n*-9), $F(2,54) = 7.6$, $p = 0.001$, and vaccenic acid (18:1*n*-7), $F(2,54) = 8.5$, $p = 0.0006$ (Table 2). Pairwise comparisons found that both MDD (−20%, $p = 0.006$) and BD (−32%, $p \leq 0.0001$) patients exhibited significantly lower DHA composition compared with controls. Both MDD (+8%, $p = 0.0018$) and BD (+14%, $p = 0.0013$) patients also exhibited significantly greater vaccenic acid composition compared with controls, and BD patients exhibited significantly greater oleic acid composition compared with controls (+10%, $p = 0.0004$). A significant main effect of group was also observed for EPA + DHA, $F(2,54) = 11.1$, $p \leq 0.0001$, and both MDD (−21%, $p = 0.006$) and BD (−31%, $p \leq 0.0001$) patients exhibited significantly lower EPA + DHA composition compared with controls. Significant main effects of group were also observed for AA:DHA, $F(2,54) = 11.6$, $p \leq 0.0001$, AA:EPA + DHA, $F(2,54) = 10.3$, $p = 0.0002$, and total n-3 (20:5 + 22:5 + 22:6)/total n-6 (18:2 + 20:3 + 20:4 + 22:4 + 22:5), $F(2,54) = 9.6$, $p = 0.0003$, ratios. Only BD patients exhibited significantly greater AA:DHA (+35%, $p \leq 0.0001$), AA:EPA + DHA (+34%, $p \leq 0.0001$), and n-6/n-3 (+23%, $p = 0.0003$) ratios relative to healthy controls. Although there was a trend for greater DHA deficits (−9%, $p = 0.09$) and elevations in the AA:DHA (+19%, $p = 0.04$) and AA:EPA + DHA (+18%, $p = 0.05$) ratios in BD patients relative to MDD patients, these were not statistically significant after correction for multiple comparisons.

3.2. Product:precursor ratios

For the 20:3/18:2 ratio (an index of delta-6 desaturase activity), there was a significant main effect of group, $F(2,54) =$

Table 2
Erythrocyte fatty acid composition.

Fatty acid [a]	HC	MDD	BD	F [b]	HC vs. MDD	HC vs. BD	MDD vs. BD
Saturated fatty acids							
Palmitic acid (16:0)	16.9 ± 0.2	17.7 ± 0.3	17.6 ± 0.4	0.158	0.03	0.12	0.89
Stearic acid (18:0)	16.4 ± 0.3	17.0 ± 0.6	16.2 ± 0.3	0.331	0.32	0.61	0.21
Monounsatutated fatty acids							
Oleic acid (18:1*n*-9)	11.9 ± 0.2	13.0 ± 0.2	13.2 ± 0.3	**0.001**	**0.0018**	**0.0013**	0.59
Vaccenic acid (18:1*n*-7)	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	**0.0006**	0.012	**0.0004**	0.12
Polyunsaturated fatty acids							
Linoleic acid (18:2*n*-6)	10.9 ± 0.3	9.5 ± 0.3	10.0 ± 0.4	0.018	0.0014	0.10	0.32
Homo-gamma-linoleic (20:3*n*-6)	1.5 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	0.065	0.71	0.02	0.11
Arachidonic acid (AA, 20:4*n*-6)	16.9 ± 0.3	16.2 ± 0.6	17.0 ± 0.4	0.417	0.31	0.87	0.29
Docosatetraenoic acid (22:4*n*-6)	4.0 ± 0.1	3.8 ± 0.2	4.1 ± 0.1	0.597	0.46	0.85	0.38
Docosapentaenoic acid (22:5*n*-6)	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.503	0.45	0.23	0.78
Eicoapentaenoic acid (EPA, 20:5*n*-3)	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.095	0.09	0.07	0.83
Docosapentaenoic acid (22:5*n*-3)	2.3 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	0.251	0.11	0.23	0.66
Docosahexaenoic acid (DHA, 22:6*n*-3)	4.4 ± 0.2	3.5 ± 0.2	3.0 ± 0.2	**0.0001**	**0.007**	**0.0001**	0.09
EPA + DHA (Omega-3 index)	4.8 ± 0.3	3.8 ± 0.2	3.3 ± 0.2	**0.0001**	**0.006**	**0.0001**	0.12
n-6/n-3 ratios							
AA:DHA	4.0 ± 0.2	5.0 ± 0.4	6.2 ± 0.4	**0.0001**	0.02	**0.0001**	0.04
AA:EPA	46.5 ± 5.2	55.1 ± 5.0	59.2 ± 5.0	0.196	0.24	0.09	0.58
AA:EPA + DHA	3.7 ± 0.2	4.6 ± 0.3	5.6 ± 0.3	**0.0002**	0.03	**0.0001**	0.05
n-6/*n*-3	7.1 ± 0.3	8.5 ± 0.8	9.2 ± 0.4	**0.0003**	0.08	**0.0003**	0.46

P-values in bold are significant after adjustment for multiple comparisons.
[a] Weight percent total fatty acids (g/100 g) expressed as mean ± S.E.M.
[b] *P*-values form the one-way ANOVA.

7.5, $p=0.0014$. Both MDD (+15%, $p=0.01$) and BD (+22%, $p=0.0004$) patients exhibited a greater 20:3/18:2 ratio relative to controls, and this ratio did not differ significantly between MDD and BD patients ($p=0.21$)(Fig. 1A). The main effect of group was not significant for either the 20:4/20:3 ratio (an index of delta-5 desaturase activity), $F(2,54)=2.2$, $p=0.11$ (Fig. 1B) or the 22:4/20:4 ratio (an index of elongase activity), $F(2,54)=0.03$, $p=0.97$. For the 18:1/18:0 ratio (an index of delta-9 desaturase activity), there was a significant main effect of group, $F(2,54)=5.4$, $p=0.007$ (Fig. 1C). BD patients (+10%, $p=0.001$), but not MDD patients ($p=0.17$), exhibited a greater 18:1/18:0 ratio relative to controls, and there was a trend for a greater ratio in BD patients relative to MDD patients (+5%, $p=0.09$). For the 22:6/22:5 ratio, there was a significant main effect of group, $F(2,54)=7.3$, $p=0.0016$. Both MDD (−15%, $p=0.04$) and BD (−28%, $p=0.0006$) patients exhibited a smaller 22:6/22:5 ratio relative to controls, and this ratio did not differ significantly between MDD and BD patients ($p=0.10$). Among all subjects ($n=55$), DHA was inversely correlated with the 20:3/18:2 ratio, $r=-0.49$, $p=0.0002$ (Fig. 1D), and positively correlated with the 20:4/20:3 ratio, $r=+0.50$, $p=0.0001$ (Fig. 1E). DHA was not correlated with the 22:4/20:4 ratio, $r=+0.01$, $p=0.93$. Erythrocyte DHA was inversely correlated with the 18:1/18:0 ratio, $r=-0.45$, $p=0.0007$ (Fig. 1F).

3.3. Gender and age

For n-3 fatty acids, there were no significant Gender × group interactions for DHA (22:6*n*-3) (Fig. 2A), $F(2,54)=0.23$, $p=0.79$, EPA (20:5*n*-3), $F(2,54)=1.0$, $p=0.36$, EPA + DHA, $F(2,54)=0.31$, $p=0.73$, DPA (22:5n-3), $F(2,54)=0.95$, $p=0.39$, or total n-3, $F(2,54)=0.30$, $p=0.74$. For n-6 fatty acids, there were no significant Gender × group interactions for linoleic acid (18:2*n*-6), $F(2,54)=0.29$, $p=0.75$, whereas trends were found for 20:3*n*-6, $F(2,54)=2.1$, $p=0.056$, and 20:4*n*-6, $F(2,54)=3.9$, $p=0.02$. The Gender × group interaction was significant for the 20:3/18:2 ratio, $F(2,54)=5.1$, $p=0.009$ (Fig. 2B). Specifically, female BD patients exhibited a greater 20:3/18:2 ratio relative to both female controls (+26%, $p=0.01$) and female MDD patients (+24%, $p=0.02$), but not male BD patients ($p=0.16$). Male MDD patients exhibited a greater 20:3/18:2 ratio relative to female MDD patients (+18%, $p=0.03$). Among all subjects ($n=55$), age was not significantly correlated with individual n-3 fatty acid compositions, including DHA, $r=+0.15$, $p=0.26$, individual n-6 fatty acids, individual monounsaturated fatty acids, or individual saturated fatty acids.

3.4. Symptom severity

The relationship between fatty acid compositions and ratios and depression (HDRS) and manic (CARS-M) symptom severity scores were determined by linear regression analysis (Table 3). After correcting for multiple comparisons, there were no significant correlations between relevant fatty acid compositions, including DHA, and depression or mania symptom severity scores.

4. Discussion

The main finding of the present study is that medication-free MDD and BD patients exhibit selective erythrocyte DHA deficits compared with demographically similar healthy controls residing in the U.S. The deficit was specific to DHA, as there were no significant deficits observed for other principal n-3 fatty acids including EPA and DPA or n-6 fatty acids including AA. Although we did not observe significant

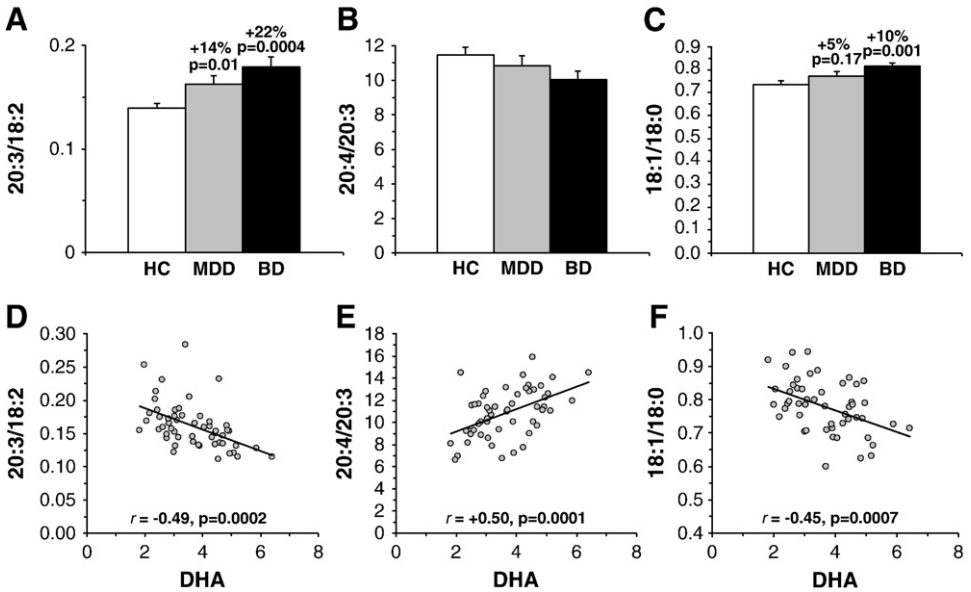

Fig. 1. Comparison of the 20:3/18:2 ratio (A), an index of delta-6 desaturase activity, and the 20:4/20:3 ratio (B), an index of delta-5 desaturase activity, and the 18:1/18:0 ratio, an index of delta-9 desaturase activity (C), in healthy controls (HC) and patients with MDD or BD. Correlations between erythrocyte DHA composition and the 20:3/18:2 ratio (D), the 20:4/20:3 ratio (E), and the 18:1/18:0 ratio (F) among all subjects ($n=55$). Percent difference from controls and associated *p*-values (two-tailed) are presented in A–C, and Pearson correlation coefficients and *p*-values (two-tailed) are presented in D–F. Values are group mean ± S.E.M.

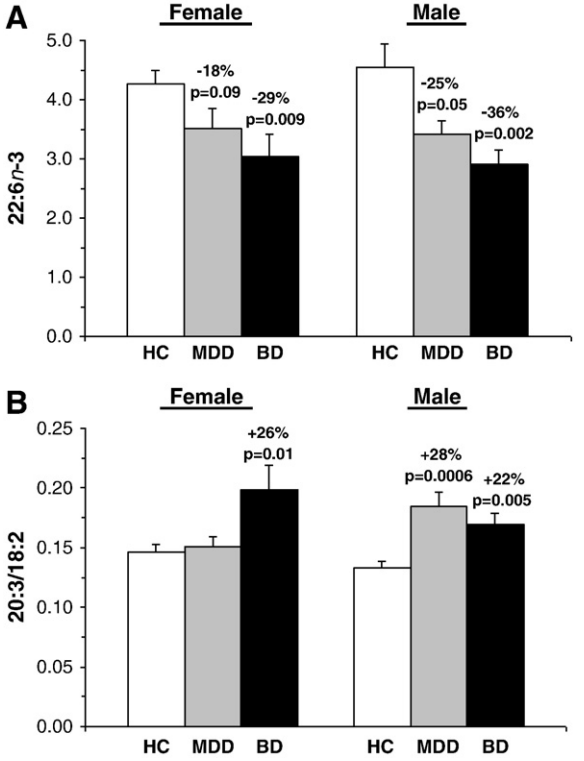

Fig. 2. (A) Comparison of erythrocyte DHA (22:6n-3) composition in male and female healthy controls (HC) and patients with MDD or BD. Note that there are no gender differences in erythrocyte DHA composition in any group. (B) Comparison of the 20:3/18:2 ratio in male and female healthy controls (HC) and patients with MDD or BD. Note that unlike female patients with BD, female patients with MDD do not exhibit a greater 20:3/18:2 ratio relative to female controls. Percent difference from controls and associated *p*-values (two-tailed) are presented. Values are group mean ± S.E.M.

Table 3
Correlations with HDRS and CARS-M scores.

	MDD		BD	
	r	*p*	*r*	*p*
HDRS				
22:6n-3 (DHA)	0.04	0.89	−0.31	0.22
20:5n-3 (EPA)	−0.11	0.69	0.09	0.71
EPA + DHA	0.02	0.94	−0.26	0.31
18:2n-6	0.26	0.38	−0.22	0.40
20:3/18:2	−0.21	0.47	0.43	0.09
20:4n-6 (AA)	−0.38	0.19	0.37	0.15
AA:EPA	−0.13	0.67	0.03	0.93
AA:EPA + DHA	−0.12	0.69	0.46	0.06
CARS-M				
22:6n-3 (DHA)	0.58	0.03	0.14	0.62
20:5n-3 (EPA)	−0.06	0.83	−0.23	0.41
EPA + DHA	0.54	0.04	0.09	0.74
18:2n-6	0.09	0.74	0.41	0.13
20:3/18:2	0.07	0.80	−0.64	0.009
20:4n-6 (AA)	−0.03	0.91	−0.12	0.66
AA:EPA	0.29	0.33	0.14	0.62
AA:EPA + DHA	−0.56	0.04	−0.13	0.65

differences between MDD and BD patients for any fatty acid, there were clear trends for greater DHA deficits, and a larger AA:DHA ratio, in BD patients compared with MDD patients. There were no significant gender differences in erythrocyte DHA composition in controls or patients with MDD or BD, and erythrocyte DHA composition was not correlated with age. Erythrocyte DHA composition was not significantly correlated with depression or mania symptom severity. The magnitude of erythrocyte DHA deficits observed in MDD (−20%) and BD (−32%) patients are comparable to those previously observed in patients residing in Asia and western Europe (Chiu et al., 2003; Edwards et al., 1998; Peet et al., 1998; Ranjekar et al., 2003), thus providing cross-national evidence for erythrocyte DHA deficits in MDD and BD. Moreover, the present finding is also consistent with our prior finding of DHA deficits in the postmortem PFC (BA10) gray matter of patients with MDD and BD (McNamara et al., 2007, 2008).

Prior studies have observed inverse correlations between DHA and/or EPA, and positive correlations between plasma n-6:n-3 ratios, principally AA:EPA, and measures of depression and/or manic symptom severity (Adams et al., 1996; Edwards et al., 1998; Conklin et al., 2007; Maes et al., 1996; Sublette et al., 2009). In the present study, neither DHA nor the AA:EPA ratio were significantly correlated with either depression or mania symptom severity scores. However, healthy subjects commonly exhibit low scores on both depression and mania scales, indicating that erythrocyte DHA levels are ultimately inversely correlated with depression and mania symptom severity. Furthermore, although these patients were drug-free up to 2 weeks prior to blood collection, carry over effects of medications on both mood and erythrocyte fatty acid composition cannot be ruled out. Nevertheless, because erythrocyte membrane-esterified DHA composition has a long half-life, free plasma fatty acid levels may be more sensitive to acute and more subtle variations in mood symptom severity (Sublette et al., 2009).

Selective reductions in dietary n-3 fatty acid intake are associated with elevations in delta-6 (*FADS2*) and delta-5 (*FADS1*) desaturase gene expression and activity in rat liver, and elevations in n-6 fatty acid biosynthesis and DPA (22:6n-6) tissue composition (Igarashi et al., 2007). In the present study, the erythrocyte 20:3/18:2 ratio, an index of delta-6 desaturase activity (Harmon et al., 2003; Obukowicz et al., 1998), was significantly elevated in patients with MDD and BD, and inversely correlated with erythrocyte DHA. Moreover, the erythrocyte 20:4/20:3 ratio, an index of delta-5 desaturase activity, and the 22:4/20:4 ratio, an index of elongase activity, did not differ between groups. Because delta-6 desaturase is the rate-limiting enzyme in the biosynthesis of n-3 and n-6 fatty acids from alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (18:2n-6), respectively (Fig. 3), pharmacological inhibition of delta-6 desaturase results in significant reductions of both DHA and AA (Harmon et al., 2003; Obukowicz et al., 1998). In the present study, however, erythrocyte AA and the down-stream n-3 fatty acid metabolites of ALA, EPA (20:5n-3) and DPA (22:5n-3), were not significantly reduced in MDD and BD patients. These data suggest that the DHA deficits observed in MDD and BD patients cannot be wholly attributed to reductions in desaturase or elongase activity. Indeed, the selective DHA deficit, reduction in the DHA/DPA (22:5n-3) ratio, and lack of

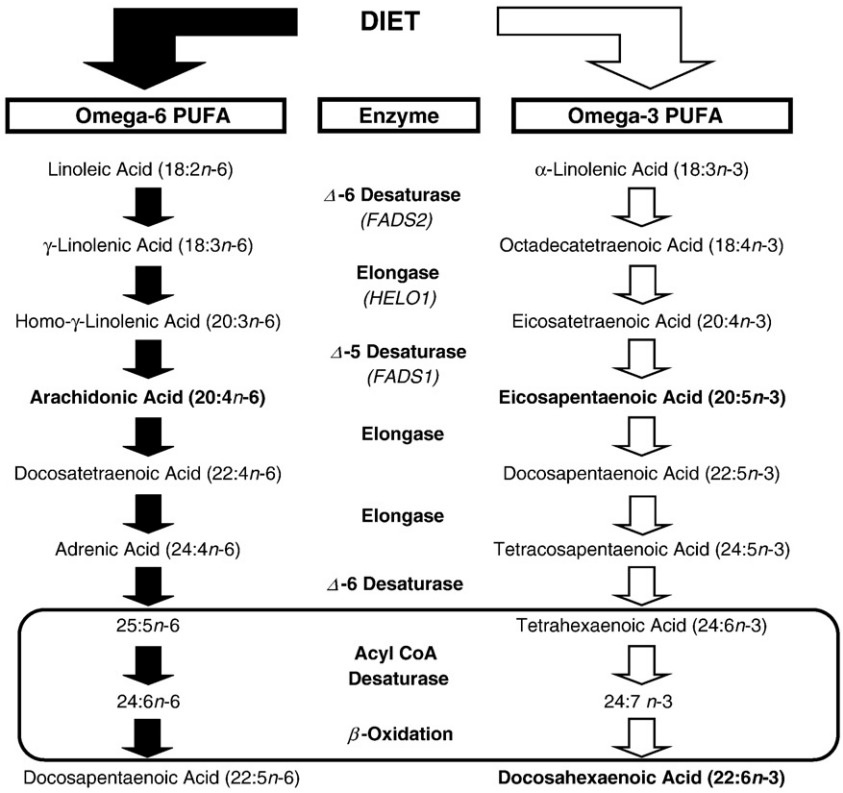

Fig. 3. Diagram illustrating the biosynthetic pathways of omega-6 and omega-3 PUFAs from dietary precursors linolenic acid (LA, 18:2*n*-6) and alpha-linolenic acid (ALA, 18:3*n*-3), respectively. The biosynthesis of long-chain fatty acid products of LA and ALA requires a series of common microsomal elongation and delta (Δ) 5- and 6-desaturase-mediated reactions, and the final synthesis of DHA and DPA (22:5n-6) requires additional enzymatic modifications within peroxisomes. It is proposed that a defect in peroxisomal function could account for the selective DHA deficits, normal levels of n-3 fatty acid precursors of DHA including EPA (20:5n-3) and DPA (22:5n-3), and lack of reciprocal elevations in 22:5n-6 in patients with MDD and BD.

a reciprocal increase in 22:5n-6 in MDD and BD patients are consistent with a defect in peroxisome function (Sprecher and Chen, 1999). This is supported by the finding that peroxisomal biogenesis disorders are also associated with erythrocyte DHA deficits, no corresponding elevation in 22:5n-6, and normal levels of n-3 fatty acid precursors of DHA (Martinez et al., 1994; Moser et al., 1999).

Delta-9 desaturase (stearoyl-CoA desaturase, SCD) mediates the biosynthesis of oleic acid (18:1) from stearic acid (18:0) (Ntambi and Miyazaki, 2004), and oleic acid is a major component of triglycerides (Attie et al., 2002). *SCD* gene expression is repressed by n-3 fatty acids at the level of transcription and mRNA stability (Ntambi, 1999), and is up-regulated in the rat liver in response to moderate and selective reductions in n-3 fatty acids (Igarashi et al., 2007). In the present study, we observed an inverse correlation between erythrocyte DHA and EPA + DHA and the 18:1/18:0 ratio, and this ratio and oleic acid composition were significantly elevated in BD patients relative to controls. Prior studies have found that the 18:1/18:0 ratio is positively correlated with triglyceride levels (Attie et al., 2002), and that regular consumption of fish (Harris, 1989) or preformed EPA + DHA (McKenney and Sica, 2007) is associated with a 20–40% reduction in plasma triglyceride levels. Although we did not determine triglyceride levels in the present study, the present findings may therefore have mechanistic implications for the significantly elevated triglyceride levels observed in BD patients (Fiedorowicz et al., 2008; Sicras et al., 2008).

There is now substantial evidence that lower erythrocyte EPA + DHA composition (the 'omega-3 index') is a preventable risk factor for coronary heart disease (Harris, 2008), and emerging findings suggest that erythrocyte EPA + DHA deficiency may contribute to the elevated rate of coronary heart disease comorbidity in MDD (McNamara, 2009a). Based on data from prospective longitudinal studies investigating sudden cardiac death as an endpoint, erythrocyte EPA + DHA composition of ≤4% was determined to be associated with a 10-fold greater risk for sudden cardiac death compared with >8% (Harris and von Schacky, 2004). In the present study, erythrocyte EPA + DHA composition in healthy controls (4.8% ± 1.1%) was similar to that previously observed in a larger cohort of healthy subjects residing in the U.S. (4.9% + 2.1%, Sands et al., 2005), and approximately one-half that observed in healthy Japanese adults (8.5 + 1.8%, Itomura et al., 2008). Furthermore, 65% of MDD patients and 78% of BD patients in the present study exhibited an erythrocyte EPA + DHA composition of ≤4.0%, compared with 25% of controls. Moreover, the mean erythrocyte EPA + DHA composition observed in MDD (3.8% + 1.6%) and BD (3.3% + 1.6%) patients resemble that observed in patients with acute coronary syndrome (3.4% + 1.6%; Block et al., 2008). These data therefore suggest that the majority of MDD and BD patients in the present study are at high risk for sudden cardiac death.

Prior preclinical evidence suggests that mood-stabilizer medications reduce AA turnover in rat brain (Rapoport et al., 2009), and we reported that AA deficits found in the postmortem PFC of medication-free BD patients were partially normalized in patients treated with mood-stabilizer medications (McNamara et al., 2008a). Furthermore, chronic lithium treatment, resulting in therapeutically-relevant plasma concentrations, was found to significantly increase rodent erythrocyte membrane AA composition (McNamara et al., 2008b). In the present study, we did not observe significant alterations in erythrocyte AA composition in medication-free BD patients, which is consistent with prior case–control studies (Ranjekar et al., 2003; Sublette et al., 2007). However, another case–control study found that medicated BD patients exhibit significant deficits in erythrocyte AA (Chiu et al., 2003). In view of these conflicting findings, and the confounding influence of mood-stabilizer medications on erythrocyte AA composition, future studies investigating erythrocyte AA composition in medication-naïve first-episode manic patients are warranted.

4.1. Implications

These findings corroborate prior case–control studies finding deficits in erythrocyte DHA composition in MDD and BD patients, and therefore provide additional evidence implicating n-3 fatty acid deficiency in the pathophysiology of recurrent affective disorders. Erythrocyte DHA composition is therefore a robust and reliable biomarker of n-3 fatty acid deficiency in patients with mood disorders. In view of the recent finding that erythrocyte DHA composition is positively correlated with functional activity in the PFC (McNamara et al., 2010), the present findings may additionally have implications for cerebral hypoperfusion observed in patients with mood disorders (Ito et al., 1996). The present data also suggest that a defect in peroxisome function may contribute to erythrocyte DHA deficits in MDD and BD. If true, this would have important implications for n-3 fatty acid interventions aimed at reversing this deficit. Specifically, preformed DHA, rather than pre-peroxisome precursors of DHA including EPA, would be required. This is supported by the findings that preformed DHA corrects erythrocyte DHA deficits in patients with peroxisomal biogenesis disorders (Martínez et al., 2000; Moser et al., 1999). Moreover, this may explain in part why supplementation with DHA in addition to EPA (Stoll et al., 1999), but not EPA alone (Keck et al., 2006), is efficacious in the treatment of mood symptoms in BD patients. Increasing erythrocyte DHA to levels observed in the Japanese population (6.8%, Itomura et al., 2008), where the lifetime prevalence rates if MDD and BD are among the lowest in the world, may represent an appropriate target for future primary and secondary prevention trials (McNamara, 2009b).

4.2. Limitations

This study has two primary limitations. First, data regarding habitual dietary n-3 fatty acid intake was not obtained to evaluate its contribution to the observed erythrocyte DHA deficits. However, as discussed above, the precursors of DHA, EPA and DPA, were not significantly altered in MDD or BD patients, suggesting comparable dietary intake. Second, data regarding cigarette smoking status was not obtained, and prior studies have observed an effect of cigarette smoking on erythrocyte DHA composition in schizophrenic patients (Hibbeln et al., 2003). However, other studies in healthy adults (Sands et al., 2005) and BD patients (Chiu et al., 2003) have not observed an association between cigarette smoking and erythrocyte DHA composition.

4.3. Conclusions

This study demonstrates that young adult patients with MDD or BD exhibit significant and selective deficits in erythrocyte DHA composition relative to healthy controls residing in the U.S. These data are consistent with the findings of fatty acid studies conducted in other countries, and add to a growing body of evidence implicating n-3 fatty acid deficiency in the pathophysiology and potentially pathogenesis of recurrent affective disorders. The present data also suggest that erythrocyte DHA deficits, and associated elevations in delta-6 and delta-9 desaturase activity, are greater in BD patients than MDD patients. The present data additionally suggest that MDD and BD may be associated with a defect in peroxisomal function, which has implications for n-3 fatty acid interventions aimed at preventing or reversing this deficit.

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Conflict of interest

No conflict declared.

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