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ORIGINAL CONTRIBUTION

N-3 PUFA supplementation alleviates anxiety symptoms by manipulating erythrocyte fatty acid levels in depression

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Abstract

Purpose Major depressive disorder (MDD) is frequently accompanied by the symptoms of clinical anxiety. Since our previous research has found that n-3 PUFA supplementation alleviates anxiety in MDD, this study was aimed to further explore whether n-3 PUFA supplementation improves anxiety symptoms in depression by directly manipulating fatty acid levels.

Methods A secondary analysis of biomarker data (erythrocyte fatty acid composition) collected as part of the randomized clinical trial which investigated the adjunctive effect of n-3 PUFAs was conducted on 72 venlafaxine-treated outpatients with first-diagnosed, drug-naïve depression. All participants with longitudinal biomarker data were included in the association analysis to determine how n-3 PUFA supplementation influences fatty acid composition and alleviates anxiety symptoms in depression.

Results Decreases of the C20:3n6 were found in all participants at both follow-up time points ($\chi^2 = 96.36$, $p = 0.000$). The n-3 index ($\chi^2 = 10.59$, $p = 0.001$), EPA ($\chi^2 = 24.31$, $p = 0.000$), and C22:5n3/C20:5n3 ratio ($\chi^2 = 10.71$, $p = 0.001$) were increased, while C22:4n6 ($\chi^2 = 7.703$, $p = 0.006$) was decreased in n-3 PUFA group compared to the placebo group. The improvement in anxiety symptoms positively correlates with the extent of reduction of C16:0, C18:0, and total fatty acid levels as well as D5 desaturase activity ($p < 0.05$).

Conclusion These data suggest that the anxiolytic effect exerted by n-3 PUFAs in first-diagnosed, drug-naïve depression is manipulated by erythrocyte fatty acid levels. Saturated fatty acid levels have an important role in predicting the severity of anxiety symptoms.

Keywords Depression · N-3 PUFAs · Fatty acids · Anxiety

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Introduction

Depression is a highly prevalent and debilitating mental disorder, recognized as one of the leading causes of disability worldwide [1]. It is frequently accompanied by anxiety [2], which has been shown to adversely impact health-related quality of life and increase the risk of all-cause mortality [3]. Around 85% of individuals with depression also experience significant symptoms of anxiety [4]. Mixed depressive and anxiety disorders were included in the depressive disorders chapter of the International Classification of Disease (ICD-11), showing that both disorders could occur together. Previous research has shown that patients with major depressive disorder and significant anxiety in the STAR*D trial had lower remission rates, longer recovery times, increased side effects, and more severe adverse events, resulting in worse outcomes compared to those without anxiety [5]. It is critical to address anxiety symptoms in patients with depression because these patients have a poor prognosis and are more treatment resistant [6].

The treatment recommendations for anxiety disorders or anxiety symptoms include psychological therapy, such as cognitive behavioral therapy and pharmacotherapy, or a combination of both [7]. Apart from the first-line drugs, such as the selective serotonin reuptake inhibitors and serotonin-norepinephrine reuptake inhibitors [8], nutritional supplements have also drawn much attention recently. Previous research has found n-3 PUFAs show mild to moderate efficacy for the treatment of depression [9, 10]. At the same time, there has been much interest in whether interventions with n-3 PUFAs can prevent or alleviate anxiety. 12-week supplementation of n-3 PUFA was found to have the ability to reduce inflammation and anxiety among healthy young adults [11]. A systematic review revealed modest anxiolytic effects of n-3 PUFAs in individuals with anxiety symptoms [12]. However, some discouraging clinical findings related to the efficacy of n-3 PUFAs in psychiatric disorders such as anxiety and depression have been gradually reported in recent years [13, 14]. To explore the key factors determining the efficacy of n-3 PUFAs, biomarker analyses as well as their correlation to clinical outcomes are required.

This is a second analysis of the randomized placebo-controlled clinical trial, which mainly investigated the efficacy of n-3 PUFAs among outpatients with first-diagnosed, drug-naïve depression. Our previous research has found that supplementation with n-3 PUFAs was more effective than placebo in relieving anxiety symptoms in venlafaxine-treated depressed patients at the beginning of treatment [15]. Apart from primary outcomes, we also studied erythrocyte fatty acid levels before and after the intervention in this study. We hypothesized that n-3 PUFA supplementation might alleviate anxiety symptoms in depressed patients by directly manipulating fatty acid levels, especially n-3 PUFAs, and an increase of n-3 PUFA levels between baseline and after-treatment would predict improvement in anxiety symptoms.

Methods

Protocol

The study utilized samples from a previous randomized controlled trial of fish oil-assisted treatment of depression, and the detailed protocol was published [15]. Patients were recruited in the Second Xiangya Hospital of Central South University from March 2017 to January 2020 and met the diagnosis of depression in the Diagnostic and Statistical Manual of Mental Disorder-V (DSM-5) with a single episode. All patients had (1) 24-item Hamilton Depression Rating Scale (HAMD) scores $\geq$ 21, (2) aged 18–50 years, and (3) first-time diagnosis and never taking antipsychotics were included and exclusion criteria are as follows: (1) other serious comorbidities and mental illness, (2) apparent suicide attempt or behavior, (3) a history of psychoactive drug abuse (excluding alcohol and tobacco), (4) daily intake of benzodiazepines, (5) electroconvulsive therapy within the past 6 months or requires it for the current episode, (6) conditions or medications potentially affecting biomarkers include: long-term regular use of NSAIDs, COX-2 inhibitors, immunosuppressants, hormone, interferons, chemotherapy, anticoagulants, malignant tumors, active autoimmune diseases and inflammatory bowel disease; (7) taking n-3 PUFA supplements or consuming fatty fish that are high in n-3 PUFAs more than twice per week. A total of 72 subjects met the inclusion criteria, and ultimately participated in the clinical trial. Participants were randomized to the placebo and n-3 PUFA groups, and all enrolled subjects were administered a therapeutic dose of venlafaxine (75–225 mg/d) with follow-up at 4 weeks and 12 weeks after treatment, respectively. All patients were required for score assessment and venous blood collection during fasting at every visit. Patients in the n-3 PUFA group took fish oil capsules 8*1 g/capsule (EPA 1440 mg/day, DHA 960 mg/day) daily for twelve weeks, and the placebo group took the same dose of soybean oil capsules. The clinical trial was approved by the Ethics Committee of the Second Xiangya Hospital of Central South University (MDD201610). All participants volunteered to participate in the study and all signed informed consents.

Clinical assessment

Demographic and clinical information including gender, age, BMI, and education were collected from each subject at baseline, and assessments of HAMD, HAMA, SAS, and BDI were completed at baseline, week 4, and week 12 of treatment.

Sample processing and analysis

The blood samples were centrifuged at 3000 rpm for 10 min, and the plasma was removed and stored in a refrigerator at -80 °C. The extraction and quantification of the fatty acid composition of the erythrocyte membrane were performed as shown in the previous study [16]. After esterification, fatty acid methyl esters were detected by Agilent 7890 A/5975 C gas chromatography-mass spectrometer and separated on a VF-23 ms column (30 m*250μm I.D., film thickness: 0.25 μm, maximum operating temperature: 260 °C). The absolute concentrations of fatty acids are expressed in mmol/L, and some of the indexes are expressed as relative contents or ratios. N-3 PUFAs are defined as C20:5n3 + C22:5n3 + C22:6n3; N-6 PUFAs are defined as C18:2n6c + C20:3n6 + C20:4n6 + C22:4n6; Total PUFAs are the sum of n-3 PUFAs and n-6 PUFAs. N-3 index (%) was calculated as the ratio of (EPA + DHA)/FAs*100; The ratio of n-6/n-3 was used to assess the balance of n-6 and n-3 PUFAs. N-6 and n-3 extension processes were assessed using the C22:4n6/C20:4n6 ratio and the C22:5n3/C20:5n3 ratio, respectively. The ratio of fatty acid products to precursors is called the desaturase index and can be used to estimate the desaturase activity. In this study, SCD-1 activity was assessed using the C18:1/C18:0 ratio; D6 desaturase activity was assessed using the C20:3n6/C18:2n6 ratio; and D5 desaturase activity was assessed using the C20:4n6/C20:3n6 ratio [17].

Table 1 Baseline characteristic of depressive patients

Parameter	n-3 PUFAs ($n = 36$)	Placebo ($n = 36$)	P value
Age	26.33 (8.07)	27.11 (8.14)	0.685
Male/Female	15/21	11/25	0.326
BMI	20.85 (3.02)	21.83 (2.88)	0.937
Education year	14.42 (2.94)	13.26 (3.21)	0.122
BDI	28.42 (9.77)	29.67 (9.10)	0.576
SAS	44.92 (7.95)	45.92 (7.18)	0.577
HAMD	29.97 (6.21)	29.50 (7.43)	0.771
HAMA	22.44 (6.21)	23.61 (6.52)	0.440

BMI: body mass index; BDI: Beck Depression Inventory; HAMA: Hamilton Anxiety Scale; HAMD: 24-item Hamilton Depression Rating Scale; SAS: Self-rating Anxiety Scale

The data are expressed as mean (standard deviation)

Statistical analysis

Data analyses were performed by IBM SPSS Statistics 26.0, GraphPad Prism 8.0, and R 4.2.1. Baseline data that conformed to the normal distribution were analyzed using independent samples t-tests, and data were presented in mean (sandard deviation), and chi-square tests or Fisher's exact probability method were used for statistical analysis of count data. Nonnormal fatty acid data were expressed as median (P25-P75), and repeated measures were analyzed using a generalized estimating equation (GEE) with Bonferroni correction for multiple comparisons. The correlation between the change in fatty acid levels and the change in scale scores was examined by Spearman's correlation analysis. All statistical significance tests were performed using two-tailed tests, and $p < 0.05$ was considered a statistically significant difference.

Results

Patient characteristic

A comparison of demographic data and clinical characteristics between the fish oil and placebo groups is shown in Table 1. There were no significant differences in age, gender, BMI, education years, and scale scores between the n-3 PUFA and placebo groups.

Erythrocyte membrane FAs after 4-week and 12-week treatment

As shown in Table 2; Fig. 1, the supplement of fish oil on C18:2n6c ($\chi^2 = 4.794$, $p = 0.029$), C20:3n6 ($\chi^2 = 8.479$, $p = 0.004$), C20:5n3 EPA ($\chi^2 = 24.31$, $p = 0.000$), C22:4n6 ($\chi^2 = 7.703$, $p = 0.006$), n-6 PUFAs ($\chi^2 = 5.910$, $p = 0.015$), n-6/n-3 PUFAs ($\chi^2 = 6.128$, $p = 0.013$), (EPA + DHA)/total FAs ($\chi^2 = 10.59$, $p = 0.001$), C22:4n6/C20:4n6 ($\chi^2 = 4.332$, $p = 0.037$), C22:5n3/C20:5n3 ($\chi^2 = 10.71$, $p = 0.001$) had a significant effect. After 4 weeks of venlafaxine treatment, levels of C20:5n3 EPA and (EPA + DHA)/total FAs increased significantly in the n-3 PUFA group, while C22:5n3/C20:5n3 and n-6/n-3 PUFAs decreased significantly ($p < 0.05$); after 12 weeks of treatment, C20:5n3 EPA continued to increase in the n-3 PUFA group, while C22:4n6 levels decreased significantly ($p < 0.05$).

After 4 and 12 weeks of treatment, C18:0 ($\chi^2 = 10.05$, $p = 0.007$), C18:1n9t ($\chi^2 = 12.76$, $p = 0.002$), C18:1n9c ($\chi^2 = 12.21$, $p = 0.002$), C20:3n6 ($\chi^2 = 96.36$, $p = 0.000$), C20:4n6 AA ($\chi^2 = 12.67$, $p = 0.002$), C20:5n3 EPA ($\chi^2 = 15.64$, $p = 0.000$), C22:4n6 ($\chi^2 = 30.39$, $p = 0.000$), total FAs ($\chi^2 = 10.73$, $p = 0.005$), total SFAs ($\chi^2 = 6.409$,

Table 2 The fatty acid levels of erythrocyte membrane between the two groups at baseline, 4-week and 12-week after treatment

	Baseline		Week 4		Week 12		intervention χ^2 (*p* value)	time χ^2 (*p* value)	interven-tion + time χ^2 (*p* value)
	n-3 PUFAs	Placebo	*n*-3 PUFAs	Placebo	*n*-3 PUFAs	Placebo			
C16:0	6.88(4.60-11.33)	8.64(5.56–12.53)	7.43(5.80-10.07)	7.29(5.91–11.66)	6.75(5.64–8.80)	7.40(5.75–10.64)	1.524(0.217)	3.248(0.197)	0.117(0.943)
C18:0	4.56(3.00-7.12)	4.80(4.04–6.39)	3.88(3.25–5.72)	4.23(3.28–7.08)	3.27(2.51–4.77)	4.44(3.36–5.90)	1.522(0.217)	10.05(**0.007**)	0.951(0.622)
C18:1n9t	5.34(3.92–7.87)	5.60(4.51–7.15)	4.84(3.45–5.45)	4.81(3.95–6.48)	3.98(2.59–6.10)[b]	5.10(3.72–5.90)	0.283(0.595)	12.76(**0.002**)	0.978(0.613)
C18:1n9c	0.18(0.08–0.35)	0.26(0.10–0.35)	0.28(0.08–0.28)	0.28(0.08–0.28)	0.12(0.07–0.22)	0.19(0.09–0.22)	0.521(0.470)	12.21(**0.002**)	1.897(0.387)
C18:2n6c	3.34(2.94–5.24)	3.81(3.02–5.28)	3.04(2.49–3.90)	3.97(3.59–5.04)	2.54(2.22–3.82)	3.79(2.73–4.68)	4.794(**0.029**)	5.321(0.070)	1.832(0.400)
C20:3n6	0.31 (0.18–0.31)	0.31 (0.25–0.31)	0.19 (0.14–0.19)[a]	0.19 (0.14–0.17)[a]	0.17 (0.10–0.17)[b]	0.17 (0.14–0.17)[b]	8.479(**0.004**)	96.36(**0.000**)	3.117(0.210)
C20:4n6AA	3.75(2.24–4.60)	4.26(2.32–5.14)	2.96(1.80–3.80)	3.35(2.64–4.17)	2.02(1.40–2.96)[b]	3.11(2.19–5.18)	3.503(0.061)	12.67(**0.002**)	3.994(0.136)
C20:5n3EPA	0.04(0.03–0.06)	0.06(0.03–0.07)	0.12(0.06–0.25)[a]	0.03(0.02–0.04)*, [a]	0.19(0.04–0.30)[b]	0.04(0.03–0.06)*	24.31(**0.000**)	15.64(**0.000**)	32.74(**0.000**)
C22:4n6	0.59(0.47–0.84)	0.81(0.40–0.92)	0.42(0.24–0.68)	0.62(0.40–0.70)	0.25(0.15–0.39)[b, c]	0.47(0.37–0.67)*	7.703(**0.006**)	30.39(**0.000**)	7.739(**0.021**)
C22:5n3	0.40(0.25–0.65)	0.59 (0.30–0.73)	0.39 (0.25–0.63)	0.40 (0.31–0.48)	0.41 (0.26–0.57)	0.44 (0.23–0.78)	0.005(0.943)	2.092(0.351)	4.343(0.114)
C22:6n3DHA	0.70 (0.49–1.17)	0.67 (0.51–0.80)	0.70 (0.49–1.17)	0.67 (0.51–0.80)	0.81 (0.46–1.01)	0.68 (0.42–1.10)	0.347(0.556)	0.163(0.922)	3.397(0.183)
Total FAs	27.66(18.70-37.93)	30.29(23.71–41.57)	24.09(19.69–32.59)	25.76(22.56–37.74)	20.98(16.75–27.06)	25.93(21.32–34.18)	2.150(0.143)	10.73(**0.005**)	0.675(0.714)
Total SFAs	11.77(7.13–17.06)	13.87(9.59–18.67)	11.50(9.11–16.35)	10.61(9.02–19.10)	10.05(8.66–13.32)	12.46(9.41–16.94)	1.583(0.208)	6.409(**0.041**)	0.255(0.880)
Total MUFAs	5.55(4.19–8.09)	5.86(4.86–7.27)	5.03(3.86–6.20)	5.10(4.15–6.87)	4.17(2.74–6.20)[b]	5.18(3.96–6.12)	0.188(0.665)	13.66(**0.001**)	1.022(0.600)
Total PUFAs	9.63(7.53–11.83)	11.04(7.11–13.69)	8.18(5.91–10.90)	8.98(8.43–11.91)	6.46(4.78–8.58)[b]	8.97(6.54–12.78)	3.797(0.051)	10.66(**0.005**)	1.532(0.465)
n-6 PUFAs	8.21(6.85–10.18)	9.50(6.66–11.81)	7.08(4.99–8.31)	8.13(7.34–10.08)	5.02(3.90–6.80)[b]	7.39(5.80-11.11)	5.910(**0.015**)	13.75(**0.001**)	2.092(0.351)
n-3 PUFAs	1.23 (0.88–2.04)	1.14 (0.86–1.29)	1.23 (0.88–2.04)	1.14 (0.86–1.29)	1.55 (0.91-2.00)	1.16 (0.81–1.77)	1.037(0.309)	0.175(0.916)	6.371(**0.041**)
n-6/n-3 PUFAs	7.48(5.88–8.66)	7.10(5.18–9.12)	5.17(3.40–6.51)[a]	7.77(6.90–9.58)*	3.60(2.93–6.19)[b]	6.67(5.07–8.41)	6.128(**0.013**)	12.09(**0.002**)	14.54(**0.001**)
UI	1.27 (1.14–1.59)	1.19 (1.11–1.49)	1.27 (1.14–1.59)	1.19 (1.11–1.49)	1.28 (1.12–1.48)	1.26 (1.11–1.52)	0.002(0.962)	2.392(0.302)	1.481(0.477)
(EPA + DHA)/total FAs	2.22 (1.89–2.99)	2.64 (1.50–3.67)	2.72 (1.39–4.33)[a]	2.08 (1.82–2.85)*	4.56 (3.23–6.24)[b]	2.15 (1.63–3.57)	10.59(**0.001**)	14.37(**0.001**)	18.98(**0.000**)
C18:0/C16:0	0.63(0.52–0.75)	0.55(0.44–0.63)	0.53(0.51–0.65)	0.59(0.50–0.65)	0.52(0.42–0.62)	0.58(0.51–0.64)	0.993(0.319)	4.142(0.126)	2.704(0.259)
C18:1n9/C18:0	1.21(1.01–1.41)	1.16(0.95–1.35)	1.21(0.98–1.43)	1.08(0.89–1.25)	1.07(0.93–1.34)	1.05(0.89–1.26)	1.393(0.238)	1.883(0.390)	0.037(0.982)
C20:3n6/C18:2n6	0.06(0.05–0.10)	0.07(0.06–0.11)	0.06(0.04–0.08)	0.04(0.03–0.06)	0.05(0.04–0.07)	0.05(0.03–0.08)	1.436(0.231)	4.122(0.127)	3.752(0.153)
C20:4n6/C20:3n6	17.70 (11.72–23.09)	17.23 (13.59–23.58)	17.70 (11.72–23.09)	17.23 (13.59–23.58)	15.22 (10.62–23.53)	18.85 (12.18–23.45)	0.053(0.818)	5.148(0.076)	2.390(0.303)
C22:4n6/C20:4n6	0.17(0.14–0.18)	0.17(0.15–0.20)	0.16(0.13–0.19)	0.17(0.14–0.20)	0.12(0.11–0.14)	0.16(0.15–0.18)	4.332(**0.037**)	5.772(0.056)	1.752(0.417)
C22:5n3/C20:5n3	11.45(7.83–16.91)	10.54(5.96–13.52)	2.52(1.53–8.58)[a]	12.44(9.28–17.01)*	2.39(1.44–6.81)	10.80(8.20–16.00)	10.71(**0.001**)	3.911(0.141)	14.87(**0.001**)

AA: arachidonic acid; DHA: docosahexaenoic acids; EPA: eicosapentaenoic acid; FAs: fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids; UI: unsaturation index

The data are expressed as median (P25-P75)

*$p < 0.05$: significantly different between n-3 PUFAs and placebo group at different visit

[a]$p<0.05$: significantly different between baseline and week 4 in the n-3 PUFAs or placebo group

[b]$p<0.05$: significantly different between baseline and week 12 in the n-3 PUFAs or placebo group

[c]$p<0.05$: significantly different between week 4 and week 12 in the n-3 PUFAs or placebo group

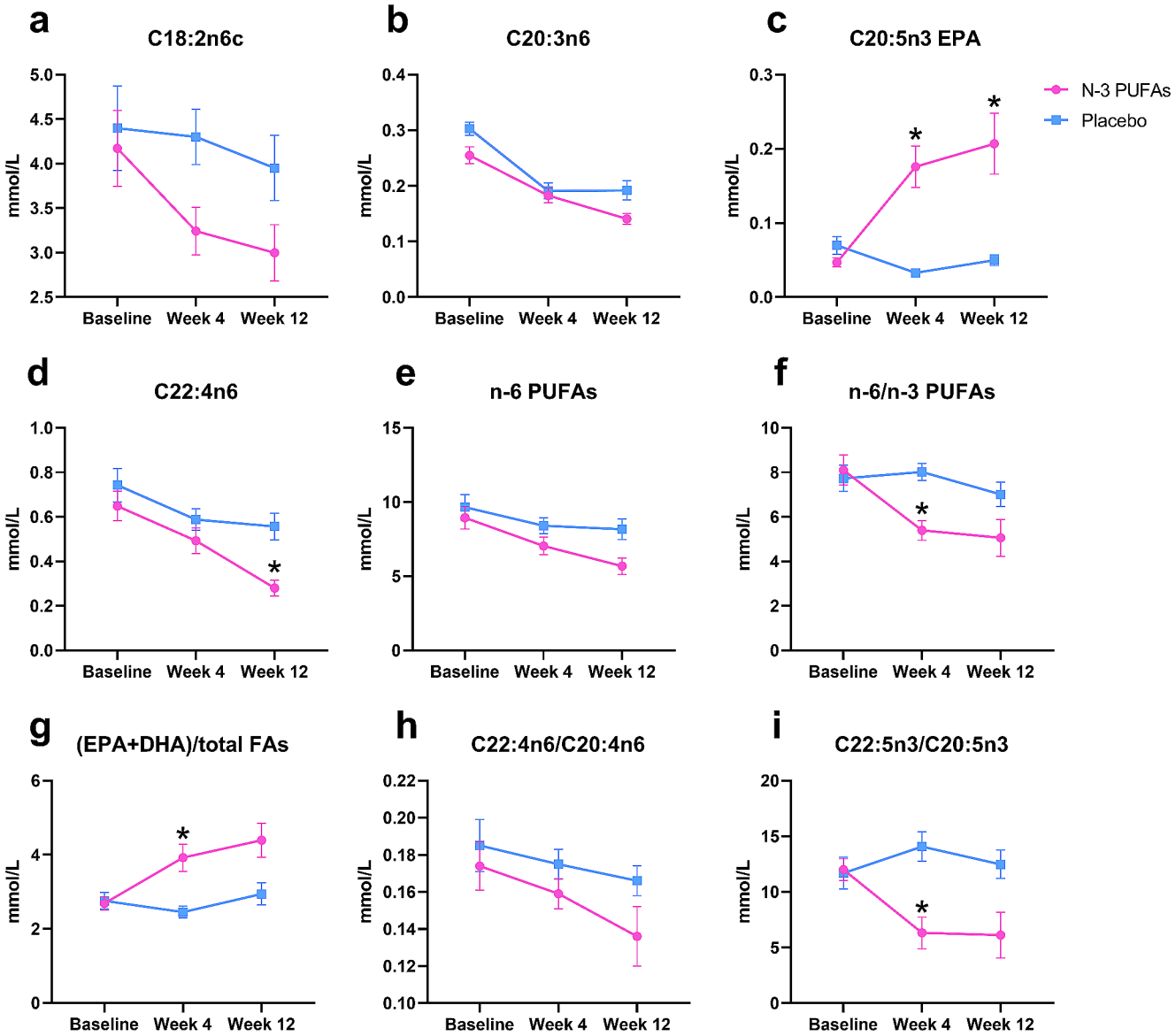

Fig. 1 Changes in fatty acid levels in the fish oil and placebo groups at the different visits
*$p < 0.05$: significantly different between n-3 PUFAs and placebo group at different visit
The data are expressed as mean (SEM)
DHA: docosahexaenoic acids; EPA: eicosapentaenoic acid; FAs: fatty acids; PUFAs: polyunsaturated fatty acids

$p = 0.005$), total MUFAs ($\chi^2 = 13.66$, $p = 0.001$), total PUFAs ($\chi^2 = 10.66$, $p = 0.005$), n-6 PUFAs ($\chi^2 = 13.75$, $p = 0.001$), n-6/n-3 PUFAs ($\chi^2 = 12.09$, $p = 0.002$) and (EPA + DHA)/total FAs ($\chi^2 = 14.37$, $p = 0.001$) levels were significantly altered. In the n-3 PUFA group, C20:3n6, n-6/n-3 PUFAs, and C22:5n3/C20:5n3 levels significantly decreased and C20:5n3 EPA, (EPA + DHA)/total FAs increased after four weeks of treatment; In the placebo group, C20:5n3 EPA and C20:3n6 decreased significantly after four weeks of treatment. In the n-3 PUFA group, C18:1n9t, C20:3n6, C20:4n6 AA, C22:4n6, total MUFAs, total PUFAs, n-6 PUFAs, n-6/n-3 PUFAs levels were significantly lower after 12 weeks of treatment compared to baseline, and C22:4n6 levels were also significantly lower compared to four weeks of treatment, while C20:5n3 EPA, (EPA + DHA)/total FAs were significantly higher; C20:3n6 was significantly lower in the placebo group.

Correlation between changes in fatty acid levels and improvement in depression and anxiety symptoms

As shown in Table 3, after 4 weeks of treatment with venlafaxine, changes in C16:0, C18:0, C18:2n6c, total FAs, total SFAs ($p < 0.05$), C20:4n6/C20:3n6 ($p < 0.01$) and

Table 3 Correlation between changes in fatty acid levels and improvement in depression and anxiety symptoms

	4-week		12-week	
	HAMA	HAMD	HAMA	HAMD
C16:0	**(0.339)** **0.018***	(0.253) 0.082	**(0.400)** **0.021***	(0.615) 0.357
C18:0	**(0.291)** **0.045***	(0.188) 0.200	(0.319) 0.071	(0.069) 0.702
C18:1n9t	(0.187) 0.204	(0.193) 0.189	(0.315) 0.074	(0.022) 0.903
C18:1n9c	(0.026) 0.863	(0.060) 0.686	(-0.016) 0.930	(-0.017) 0.696
C18:2n6c	**(0.303)** **0.037***	(0.178) 0.225	(0.191) 0.286	(0.062) 0.734
C20:3n6	(-0.125) 0.396	(-0.197) 0.180	(-0.230) 0.199	(-0.074) 0.681
C20:4n6AA	(0.167) 0.265	(0.073) 0.621	(0.029) 0.871	(0.215) 0.230
C20:5n3EPA	(0.030) 0.839	(0.147) 0.317	(0.085) 0.639	(-0.295) 0.095
C22:4n6	(0.086) 0.561	(0.012) 0.934	(0.032) 0.859	(0.279) 0.116
C22:5n3	(0.045) 0.759	(0.076) 0.606	(0.302) 0.088	(0.149) 0.407
C22:6n3DHA	(0.058) 0.694	(0.064) 0.666	(0.209) 0.244	(-0.022) 0.904
Total FAs	**(0.294)** **0.042***	(0.229) 0.118	**(0.374)** **0.032***	(0.113) 0.532
Total SFAs	**(0.360)** **0.012***	(0.274) 0.059	**(0.345)** **0.049***	(0.088) 0.624
Total MUFAs	(0.183) 0.212	(0.192) 0.190	(0.317) 0.072	(0.058) 0.749
Total PUFAs	(0.224) 0.127	(0.173) 0.240	**(0.348)** **0.047***	(0.140) 0.436
n-6 PUFAs	(0.245) 0.093	(0.151) 0.305	(0.323) 0.067	(0.138) 0.444
n-3 PUFAs	(0.025) 0.865	(0.057) 0.700	(0.172) 0.340	(-0.006) 0.973
n-6/n-3 PUFAs	(0.116) 0.433	(0.111) 0.454	(-0.009) 0.960	(0.037) 0.839
UI	(-0.283) 0.052	**(-0.331)** **0.021***	(0.031) 0.865	(0.228) 0.202
(EPA + DHA)/total FAs	(-0.216) 0.141	(-0.239) 0.101	(-0.139) 0.439	(-0.059) 0.745
C18:0/C16:0	(-0.038) 0.799	(-0.236) 0.106	(0.082) 0.650	(0.026) 0.884
C18:1n9/C18:0	(-0.133) 0.369	(0.059) 0.691	(0.195) 0.276	(0.067) 0.711
C20:3n6/C18:2n6	(-0.223) 0.128	(-0.170) 0.249	(-0.187) 0.298	(-0.152) 0.399
C20:4n6/C20:3n6	**(0.408)** **0.004****	(0.219) 0.136	(0.210) 0.240	(0.054) 0.764
C22:4n6/C20:4n6	(-0.137) 0.354	(-0.095) 0.522	(0.119) 0.509	(0.113) 0.531
C22:5n3/C20:5n3	(0.117) 0.428	(-0.230) 0.115	(0.289) 0.103	(0.295) 0.096

AA: arachidonic acid; DHA: docosahexaenoic acids; EPA: eicosapentaenoic acid; FAs: fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids; UI: unsaturation index

The data are expressed as r (*p* value)

changes in HAMA score showed positive correlations, while changes in UI and HAMD score showed a significant negative correlation ($p < 0.05$). After 12 weeks of treatment, changes in C16:0, total FAs, total SFAs, total PUFAs and changes in HAMA score showed significant positive correlation ($p < 0.05$).

Correlation between changes in fatty acid levels and changes in symptom factor scores for depression and anxiety.

As shown in Fig. 2, after 4 weeks of treatment, changes in psychic anxiety were positively correlated with C16:0, C18:2n6c, total FAs, total SFAs ($p < 0.05$). Changes in C22:4n6 /C20:4n6 were significantly positively related to somatic anxiety and psychic anxiety. After 12 weeks of treatment, changes in C16:0, C18:0, C18:1n9t, C20:4n6 AA, C22:4n6, total FAs, total SFAs, total PUFAs ($p < 0.05$) and changes in somatic anxiety showed significant positive correlations. Moreover, changes in C22:4n6 and C22:5n3/C20:5n3 were positively correlated with psychic anxiety.

Discussion

Our study investigated the change of erythrocyte fatty acid composition after venlafaxine alone or adjunct with n-3 PUFAs in depression, and also explored the correlation between the change of fatty acid levels and clinical characteristics. The results suggest that treatment with venlafaxine alone reduces EPA levels at week 4, and consistently lowers the level of C20:3n6. And n-3 PUFA adjuvant venlafaxine therapy reduces almost all types of fatty acid except n-3 PUFAs, while increasing the n-3 index in the erythrocyte membranes of depressed patients. Moreover, correlation analysis revealed no significant associations between n-3 PUFA membrane levels and any clinical outcome measures. While the amelioration of anxiety symptoms is positively correlated with changes in SFA and total fatty acid levels as well as D5 desaturase activity.

In the clinical trial, erythrocyte membrane fatty acid levels have the tendency to decrease in all treated depressed patients, and n-3 PUFA supplementation reduced most fatty acid levels in the erythrocyte membranes of depressed patients. Our previous study has found that most fatty acid levels were elevated in the erythrocyte membranes of patients with severe depression [18]. In this follow-up study, we further found that erythrocyte membrane fatty acid levels tended to be "normalized" after treatment, suggesting that erythrocyte membrane fatty acid levels have the potential to predict the efficacy of antidepressant therapy. Evidence from basic and clinical studies suggests that antipsychotics may enhance the biosynthesis of fatty acids, especially PUFA and SFA [16, 19], whereas antidepressants,

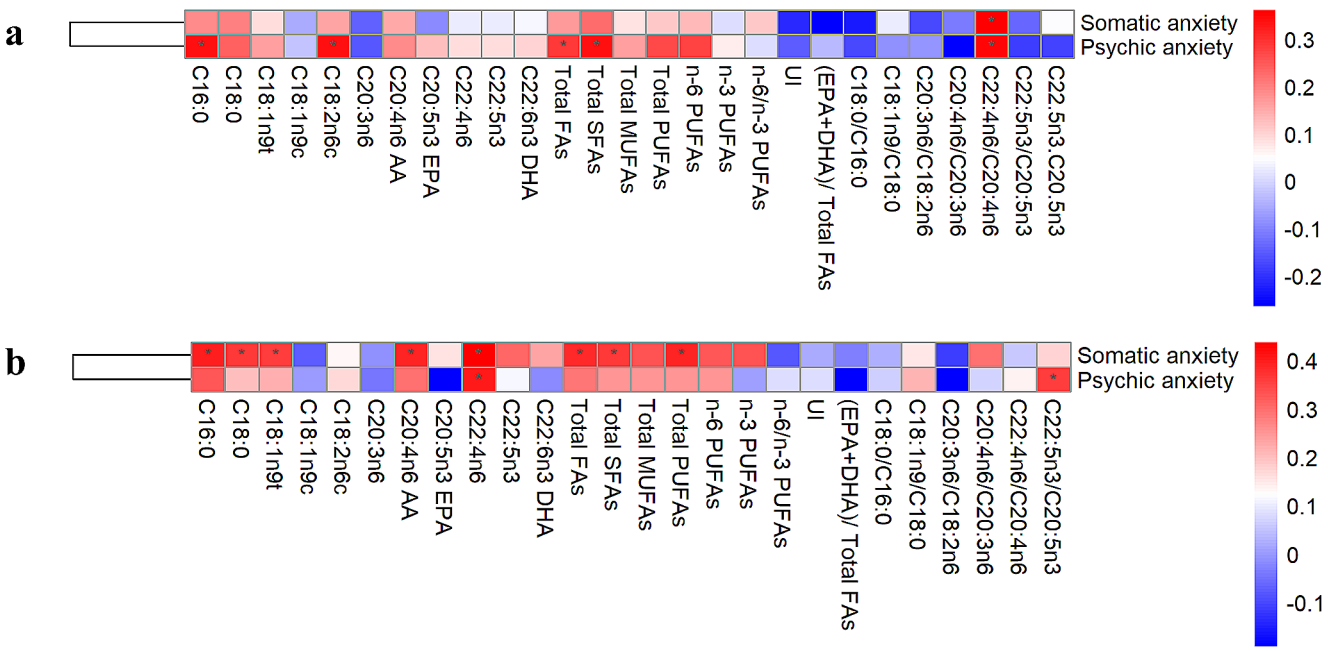

Fig. 2 Correlation of changes in fatty acid levels after treatment of 4 weeks (**a**) and 12 weeks (**b**) with HAMA scale factors
*: $p < 0.05$, AA: arachidonic acid; DHA: docosahexaenoic acids; EPA: eicosapentaenoic acid; FAs: fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids; UI: unsaturation index

particularly venlafaxine, appear to have the opposite effect on fatty acids [20]. Recent studies have found that long-term administration of fluoxetine alters the lipid composition of the macaque brain, with a major trend towards lower PUFA [21] and a reduction in plasma C20:4n6 levels [22]. The above results suggest that overall antidepressants may reduce fatty acid levels in humans, which is consistent with the present study's findings. Additionally, many fatty acids in the body are metabolized and synthesized in the liver, and venlafaxine treatment may affect liver function [23], resulting in reduced fatty acid synthesis. On the contrary, in other studies, many antidepressants were proven to activate the sterol-regulatory element binding proteins (SREBP) system with subsequent up-regulation of the downstream fatty acid biosynthesis in human glial cells [24]. Additionally, psychiatric medications, including antidepressants and antipsychotics, also cause substance-induced anxiety disorder, indicating that many underlying biological mechanisms are at work beneath the surface of anxiety.

The anxiolytic effect of fish oil appears to be through indirect modulation of the levels of other fatty acids, rather than a direct modulation effect on n-3 PUFA levels. Correspondingly, the improvement in anxiety symptoms positively correlates with the extent of reduction of C16:0, C18:0, and total fatty acid levels as well as D5 desaturase activity. Even though there was a relationship between anxiety symptoms and fatty acids at weeks 4 and 12, fish oil only improved venlafaxine's anxiolytic effects at week 4 in the current study. All participants received venlafaxine, which is routinely offered as a first-line treatment for patients with anxiety symptoms. Despite its slow onset of action, this treatment option still has a powerful anti-anxiety effect. It's possible that venlafaxine was effective enough to create a ceiling effect at week 12, beyond which n-3 PUFAs couldn't provide any more benefits. If so, then it would explain the discrepancy between clinical efficacy and biomarker analysis at week 12. The anxiolytic effect of fish oil appears to be through indirect modulation of the levels of other fatty acids, rather than a direct modulatory effect on n-3 PUFA levels. Previous research has also found that SFA increases amygdala-based serotonin metabolism and causes anxiety-like behavior in mice [25], and SFA is proven to be the principal cause of anxiety-like behavior in diet-induced obese rats [26], which is consistence to our findings. In addition, the negative trial findings in depressive symptoms were conclusively corroborated by biomarker analyses that revealed no significant associations between fatty acid levels and depressive symptoms. However, other studies have found an increase in erythrocyte n-3 PUFA levels or n-3/n-6 PUFA ratio predicted better both clinical and functional outcomes [27, 28]. These inconsistent findings between different studies might be caused by the difference from the population, baseline n-3 PUFA levels, treatment course, adherence and so on.

The present study also found that n-3 PUFA adjunctive venlafaxine promoted the decrease of n-6 PUFA levels, n-6/n-3 PUFA ratio, and C22:5n3/C20:5n3 ratio which reflects n-3 PUFA extension processes. On the other hand, this

supplementation induced increases in EPA levels and the n-3 index. This finding is consistent with previous findings [29]. Su et al. have found that 12-week supplementation of n-3 PUFA with EPA, but not DHA, significantly increased the levels of PLA2 gene expression in peripheral blood mononuclear cells in depressed patients [30]. Therefore, n-3 PUFAs may reduce fatty acid levels in vivo by regulating PLA2 which is a group of enzymes that hydrolyze phospholipids to yield fatty acids and lysophospholipids. However, increased PLA2 expression seems to be detrimental in depressed patients because PLA2 promotes the release of C20:4n6 (AA), which has pro-inflammatory effects. In addition, it has been found that n-3 PUFA supplementation may regulate fatty acid levels in vivo by decreasing hepatic SREBP levels [29, 31]. The levels of fatty acid are influenced by multifactorial effects, such as gender, lifestyle, intake, activity of metabolic enzymes, and genetic background. Therefore, more related factors should be included in future studies, and it is worthwhile to look into the mechanisms underlying them.

There are some limitations in the study. The single episode of depression which is firstly diagnosed still cannot exclude the possibility of bipolar depression due to their relatively small age. In this study, five participants were lost during the follow up, since they turned to manic episode during the treatment period. This biomarker analysis has several limitations, including the small sample size and the relatively high dropout rates of the clinical trial. What's more, some confounding factors, such as dietary habits, activity, and metabolic state which may have an impact on fatty acid metabolism are not considered.

Conclusion

In summary, the anxiolytic effect exerted by n-3 PUFAs in individuals with first-diagnosed, drug-naïve depression is not fully attributable to the regulation of n-3 PUFA levels. Rather, it seems that other indirect biological mechanisms mediate these effects. Interestingly, the role of saturated fatty acids has emerged as a significant factor in this context. Elevated levels of saturated fatty acids may be a key determinant in predicting the severity of anxiety symptoms in these patients. What's more, n-3 PUFA supplementation have an influence on n-6 PUFA level and n-3 PUFA extension processes, although their changes on clinical symptoms is unknown. This research highlights the intricate relationship between various dietary fat types and mental health and the need for a more comprehensive understanding of the role that nutrition plays in psychiatric diseases.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Human rights statement and informed consent The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the Second Xiangya Hospital of Central South University (MDD201610, 2017/06/23). Informed consent was obtained from all subjects involved in the study.

Conflict of interest The authors declare no conflict of interest.

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