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Omega-3 and omega-6 fatty acid levels in depressive and anxiety disorders

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ABSTRACT

Background: Blood levels of polyunsaturated fatty acids (PUFAs) have been associated to current depression. However, it is unclear whether this association extends to remitted depression and to anxiety disorders. This study examined the relationship of PUFAs with the presence and clinical characteristics of depressive and anxiety disorders.

Methods: Cross-sectional data was used from the Netherlands Study of Depression and Anxiety, including persons with current pure depressive disorder (n = 304), current pure anxiety disorder (n = 548), current comorbid depressive and anxiety disorder (n = 529), remitted depressive/anxiety disorder(s) (n = 897), and healthy controls (n = 634). Clinical characteristics included severity, subtypes, age of onset, duration of depression and anxiety and antidepressant use. Absolute values of omega-3 (N-3) and omega-6 (N-6) PUFAs and relative measures (as ratio of total Fatty Acids: the N-3:FA and N-6:FA ratio) in plasma were assessed using a nuclear magnetic resonance platform.

Results: Compared to controls, current comorbid depressive and anxiety disorder patients had lower N-3 PUFA levels (Cohen's d = 0.09, p = 0.012), and lower N-3:FA ratios (p = 0.002, Cohen's d = 0.11) as did current pure depressive disorder patients (Cohen's d = 0.13, p = 0.021), whereas N-6 PUFA levels were not different. No differences in PUFA levels were found between remitted patients and controls. Within patients, lower N-3 PUFA levels were only associated with higher depression severity (Beta = −0.42, p = 0.023), whereas for N-6 PUFA levels and other clinical characteristics no clear association was observed. PUFA alterations were not associated with pure anxiety.

Conclusion: It can be concluded that patients with a current depressive episode (especially the more severe cases with comorbid anxiety) have circulating N-3 PUFA levels lower than those in remission and healthy controls. No relationship was detected for N-6 PUFA levels.

1. Introduction

The impact of polyunsaturated fatty acids (PUFAs) on health are well described. Omega-3 (N-3) PUFAs consist of e.g. α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and can be mainly found in fatty fish, some other seafood, and some nuts and seeds (James et al., 2000; Simopoulos, 1999). Some randomized controlled trials have shown that intake of N-3 PUFAs ameliorate or even prevent physical illnesses such as inflammatory (Giudetti and Cagnazzo, 2012; Simopoulos, 2002) and cardiovascular diseases (La Rovere and Christensen, 2015; Simopoulos, 1999), while others have not (Hoogeveen et al., 2014; Kromhout et al., 2010). Omega-6 (N-6) PUFAs consist of e.g. linoleic and arachidonic acid which are for example found in plant and vegetable seeds and oils, as found in margarines and many processed foods (James et al., 2000; Simopoulos, 1999). High N-6 PUFA intake has been associated with chronic inflammatory diseases, cardiovascular diseases, obesity, rheumatoid arthritis, and Alzheimer's disease (Patterson et al., 2012). Low levels of N-3 and high levels of N-6 PUFAs have also been associated with neuropsychiatric disorders like depression and anxiety (Hibbeln and Salem, 1995).

Several potential biochemical mechanisms could explain the association between PUFAs and depression (Smith et al., 2011). The anti-inflammatory property of N-3 PUFAs may mitigate the overactive immune system associated with depression (Young and Conquer, 2005). Furthermore, a decrease in dietary DHA was related to a decrease in cortical serotonin and dopamine (Young and Conquer, 2005), and these neurotransmitters have been implicated in the etiology of depression

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(Smith et al., 2011; Young and Conquer, 2005). Fatty acids are implicated as regulators of gene transcription within the central nervous system (Alessandri et al., 2004; Smith et al., 2011) and may play a role in neural membrane fluidity and receptor binding (Owen et al., 2008; Smith et al., 2011; Stahl et al., 2008). For instance, DHA can affect neurological function by modulating neurotransmission, neurogenesis, myelination and more (Weiser et al., 2016).

Reviews and meta-analyses of observational studies have shown significantly lower N-3 PUFA blood levels in depressed individuals as compared to non-depressed individuals (Lin et al., 2010; Smith et al., 2011; Wani et al., 2015). However, most meta-analyses and reviews had small sample sizes (ranging from 10 to 118 depressed patients in individual studies (Lin et al., 2010)) and depression assessments between studies were diverse, from self-report questionnaires and clinical interviews to antidepressant prescriptions (Smith et al., 2011). There have been some small-scaled observational studies that found that higher n-3 PUFA blood levels were associated with lower depression severity (Adams et al., 1996; Edwards, 1998; Liu et al., 2013), especially those taking antidepressants (Féart et al., 2008), while no association with duration of depression has been found (Peet et al., 1998). Less is known about the role of clinical characteristics such as age of onset and recency of symptoms as these have never been studied in observational studies. Examining the difference in N-3 PUFA levels between current and remitted patients may help to clarify whether the association with for example N-3 PUFA is “state”-dependent (only present during an active episode and reversible after remission), or whether N-3 PUFA alterations may represent a constant underlying “trait” of depression. Although the relationship between N-6 PUFAs and depression have received much less attention, some found higher levels related to higher severity of depressive symptoms (Smith et al., 2011).

Since comorbidity of anxiety in depressive disorders is high, an association between anxiety and PUFAs is expected (Ross, 2009), however, much less examined. A recent study showed that participants with depression and comorbid anxiety had even lower N-3 PUFAs levels than depressed patients without comorbid anxiety(Liu et al., 2013). Lower N-3 PUFA levels have been found in social phobia (Green et al., 2006), but studies on other anxiety disorders are lacking. Others detected a linear relationship between N-3 PUFA intake and anxiety, with lower DHA intake being associated with a higher likelihood of anxiety (Jacka et al., 2012). This may indicate the possibility of additional anxiogenic impact of N-3 PUFAs deficiency.

Although numerous intervention studies with large sample sizes show that N-3 PUFA supplementation may have beneficial effects on depression (Appleton et al., 2015; Bloch and Hannestad, 2012; Grosso et al., 2014), large heterogeneity has been found in effect sizes. Some meta-analytic evidence (Appleton et al., 2010; Bloch and Hannestad, 2012), suggests a potential role of clinical depression characteristics in the efficacy of N-3 PUFA supplementation. Studies including more severe patients tend to show higher efficacy. However, others that not found this have suggested a role of depression subtypes, although they did not further speculate on which subtypes (Grosso et al., 2014).

To address the abovementioned issues, our primary aim is to examine the cross-sectional association of our primary outcomes N-3 and N-6 PUFA levels (both in absolute values and their ratio’s with total fatty acids), and our secondary outcomes DHA (both absolute and its ratio) and the N-6:N-3 PUFA ratio with remitted or current depressive and anxiety disorders, allowing us to be one of the first to examine whether there is a “trait”- or “state”-dependency of PUFA alterations and whether this is present in both disorders. It is hypothesized that N-3 measures (e.g. N-3 PUFA, N-3:FA ratio, DHA and DHA:FA ratio) will be lowest and N-6 PUFA measures (e.g. N-6 PUFA and N-6:FA) and the N-6:N-3 PUFA ratio will be highest going from persons with current depressive/anxiety disorders, to those with remitted depressive/anxiety disorders to healthy controls. In addition, our second aim is to explore whether specific clinical characteristics that often influence treatment response (severity, subtype, age of onset, duration and antidepressant use) can further differentiate patients with the largest PUFA differences, both for understanding the underlying mechanisms that link PUFA levels and psychiatric conditions, as well as for targeting the most appropriate patient group for future supplementation studies. It is hypothesized that those characteristics that indicate a worse disease course (higher severity, earlier age of onset, longer duration, use of antidepressants) are associated with unfavorable PUFA levels (lower N-3 PUFA levels and N-3:FA ratios and higher N-6 PUFA levels and N-6:FA ratios)(Driscoll et al., 2005; Henkel et al., 2006; Katon et al., 2010). We believe that our study has incremental value as to our knowledge this is one of the largest observational studies to date linking the presence and clinical characteristics of depressive as well as anxiety disorders to blood levels of PUFAs.

2. Materials and methods

2.1. Study sample

Participants were selected from the Netherlands Study of Depression and Anxiety (NESDA), a longitudinal observational cohort study (Penninx et al., 2008). Between 2004 and 2007 in total 2981 participants aged between 18 and 65 years were recruited from the Dutch general population (19%), primary health care (54%) and specialized mental health care (27%). The research protocol was approved by ethics committees of participating universities. All respondents provided written informed consent. Exclusion criteria were a poor comprehension of the Dutch language, and having a primary clinical diagnosis of psychotic disorder, obsessive–compulsive disorder, bipolar disorder or severe addiction disorder. At baseline, participants provided blood samples (after instructions for overnight fast) and underwent a psychiatric interview. In total 69 individuals (2.3%) had no complete blood measurements and were excluded. Presence of DSM-IV diagnoses of depressive disorders (major depressive disorder or dysthymia) and anxiety disorders (social phobia, generalized anxiety disorder, panic disorder and/or agoraphobia) was ascertained using the Composite International Diagnostic Interview (CIDI, version 2.1) administered by trained researchers. For this study, we studied five groups: patients with a current (within the past month) pure depressive disorder (n = 329), pure anxiety disorder (n = 548), comorbid depressive/anxiety disorder (n = 529), remitted (lifetime but not current) depressive and/or anxiety disorder (n = 897), and healthy controls (no lifetime disorders; n = 634).

2.2. PUFA assessment

The fatty acids measured are esterified fatty acids stemming from the lipoprotein particles, so these are not free fatty acids in the plasma but rather bound within cholesteryl esters, triglycerides and phospholipids inside the lipoproteins particles. The fatty acids were assessed in EDTA plasma samples which were collected and stored at −85 °C for later assessment. Blood samples were shipped in 2 batches (April and December 2014, further referred to as metabolic assessment wave 1 and 2, respectively). Among other metabolites, PUFA levels were quantified at 22 °C using a commercially available high-throughput proton Nuclear Magnetic Resonance (NMR) metabolomics platform (Nightingale Health Ltd., Helsinki, Finland) (Soininen et al., 2015). NMR is complementary to traditional techniques such as mass spectrometry (MS) and gas-chromatography. While NMR has a lower sensitivity, it became the gold-standard method for high-throughput metabolomics, allowing a reliable quantification of a large panel of low-molecular–weight metabolites and lipid molecules in large-scale studies (Soininen et al., 2015). In Nuclear Magnetic Resonance, the total concentration of N-3 PUFA is quantified based on a spectral signal that is arising from all fatty acids containing the N-3 double bond, and therefore N-3 PUFA is not calculated as a sum of known concentrations of individual N-3 PUFAs. The DHA signal is quantified separately from a

specific NMR signal unique to this molecule. The resolution of high-throughput NMR does currently not allow sufficient resolution for robust, independent quantification of EPA due to spectral overlap. Cross-platform biomarker confirmation for the NMR platform is given elsewhere (Würtz et al., 2015). Representative intra- and interassay coefficients of variance for analytic variation (i.e., measurement error) given for N-3 PUFA (2.7%), N-6 PUFA (4.5%) and DHA (2.7%) can be found in the supplement of a recent study (Kettunen et al., 2016).

Our primary outcomes N-3 and N-6 PUFAs were reported in absolute molar units (in mmol/L) and as a relative value (percentage of total fatty acids: N-3:FA and N-6:FA ratio). The latter has been considered biologically more informative, because this reflects PUFA levels in relation to overall FA levels(Willett, 2013). As prior studies have been inconsistent in whether they report absolute or relative levels (Smith et al., 2011), and these measures were not always highly correlated, we decided to analyze both absolute as well as relative values in relation to psychopathology. Within one individual, a high absolute level does not have to be accompanied by a high relative value, because the relative value is also determined by levels of other FA's. Consequently, in analysis, a significant association with an absolute value does not imply that also a significant association with a relative value will be found, and vice versa. Beyond the scope of our primary outcomes, we considered three additional measures of PUFAs (previously and further referred to as secondary outcomes) in post-hoc analyses: 1) the N-6:N-3 PUFA ratio, occasionally proposed by some studies as an informative index (Simopoulos, 2002), although criticized by others, and 2) DHA (mmol/l and as percentage of total fatty acids), one of the major components of N-3 PUFAs that has been linked to depression previously (Jacka et al., 2012).

2.3. Clinical characteristics

Severity of depressive symptoms was based on the 30-item self-report Inventory of Depressive Symptomatology (IDS-SR_{30}, ranging from 0 to 84) questionnaire, with higher scores indicating higher severity (Rush et al., 1996). Severity of anxiety symptoms was determined using the 21-item self-report Beck Anxiety Inventory (BAI, ranging from 0 to 63), with higher scores indicating higher severity (Beck et al., 1988). The 15-item self-report Fear Questionnaire (FQ, ranging from 0 to 120) was used to measure phobic avoidance with a higher score indicating more phobic avoidance (Marks and Mathews, 1978). The type of depressive disorder measured by the CIDI were major depressive disorder (MDD), dysthymia and their comorbidity. Additionally, three subtypes of MDD were previously derived from latent class analysis using 16 depressive symptoms from the CIDI and the IDS, and were labelled as severe melancholic MDD, severe atypical MDD and moderate MDD. A detailed description can be found elsewhere (Lamers et al., 2010). Roughly, the severe melancholic subtype is characterized by less sleep and reduced appetite while the severe atypical MDD subtype is mainly characterized by the opposite. Subtypes of anxiety as measured by the CIDI were social phobia, generalized anxiety, panic disorder, agoraphobia, or more than one anxiety disorder. Duration of depressive and/or anxiety symptoms were assessed using the Life Chart Interview (LCI), which uses a calendar method to determine life events during the past four years to refresh memory followed by assessment of symptoms in this period. Age of onset of the first affective episode was established using the CIDI. Antidepressant medication (tricyclic antidepressants (TCA)), selective serotonin reuptake inhibitors (SSRI's) and other antidepressants) was recorded based on container inspection.

2.4. Covariates

Age, gender, and years of education were obtained at baseline. Blood sampling variables were fasting status at time of blood withdrawal, sample collection site (Amsterdam, Leiden, or Groningen), and metabolic assessment wave (first or second). Missing values on fasting status (n = 6, 0.2%) were replaced by 'not fasting'. Smoking status (current, former, or never) and alcohol consumption (number of glasses of alcohol per week) were measured. Level of physical activity was measured using the total Metabolic Equivalent of Task (MET) score derived from the International Physical Activity Questionnaire (Craig et al., 2003). A higher score indicated higher amounts of calories burned due to physical activity per week. To reduce the impact of missing values for continuous covariates missing values were replaced by the overall means of the concerning variables (n = 39 (1.3%) for alcohol consumption and n = 188 (6.4%) for total MET-score). Body Mass Index was calculated as weight(kg)/length(m)^2. Self-reported somatic diseases included diabetes mellitus, heart disease (cerebrovascular disease, coronary heart disease, congestive heart failure, arrhythmia, or other) and the number of other chronic somatic diseases (thyroid disease, osteoarthritis, intestinal disorders, liver disease, cancer, neurological conditions, and allergies). As we expected that diagnosis of diabetes (Mahendran et al., 2013) and heart disease (Würtz et al., 2015) would have stronger associations with PUFA's, we separated these from other chronic somatic diseases. Use of lipid-modifying drugs (i.e. statins) and use of N-3 PUFA fish oil supplements were derived from drug container inspection and were considered as these could affect PUFA levels (Würtz et al., 2016).

2.5. Statistical analysis

Differences in characteristics across the five disorder status groups were tested using Chi-square tests, one-way analyses of variance and Kruskall-Wallis H tests. A Spearman correlation matrix was made to show the correlations between all PUFA measures. For subsequent analyses, the primary outcomes N-3 PUFA, the N-3:FA ratio, the N-6:FA ratio, and the secondary outcomes DHA and the DHA:FA ratio were log-transformed due to skewed distributions. Means and confidence intervals were back-transformed for presentation purposes.

Analyses of covariance (ANCOVA's) were performed to examine differences in the primary outcomes (N-3 PUFA, N-3:FA ratio, N-6 PUFA, N-6:FA ratio) between the disorder status groups, while controlling for possible confounders. Model 1 corrected for socio-demographic and blood sampling variables and model 2 additionally corrected for lifestyle factors and somatic health. We used False Discovery Rate (FDR) correction for multiple testing. Effect sizes comparing patients with controls were calculated as Cohen's d ($Mean_1$–$Mean_2/SD_{pooled}$). Comparisons between individual psychiatric disorder status groups were made using Šidák correction for multiple comparisons in case a significant difference for the overall psychiatric status variable was found in the ANCOVA.

To explore the presence of a dose-response association of PUFA measures with depression and anxiety symptom severity in the total sample, we plotted the fully adjusted mean severity scores (IDS, BAI and FQ) for different PUFA levels. These figures were made in SigmaPlot (Systat Software, San Jose, CA). FDR correction for multiple testing is used.

Subsequently, in exploratory analysis associations between clinical characteristics of depression and anxiety and PUFA levels were examined in subjects with a current depressive and/or anxiety disorder using fully adjusted linear regression analyses. Standardized beta's (further referred to as 'beta's') were reported. The significance level was set at 0.05. All analyses were conducted using IBM SPSS statistics software, version 22 (IBM Corp., Armonk, NY,USA).

In additional analyses, we performed a similar ANCOVA analyses for our secondary outcomes DHA, DHA:FA and N-6:N-3 PUFA ratio. FDR method for correction for multiple testing was used.

Table 1
Characteristics of the study sample.

	Controls (n = 634)	Remitted depressive or anxiety disorder (n = 897)	Current pure depressive disorder (n = 304)	Current pure anxiety disorder (n = 548)	Current comorbid depressive and anxiety disorder (n = 529)	P-value
Sociodemographic variables						
Age in years, mean (SD)	41.3 (14.6)	42.5 (13.0)	42.3 (12.5)	41.6 (12.6)	42.0 (11.8)	.48
Female, yes%	61.8	69.7	64.1	67.0	67.3	0.01
Years of education, mean (SD)	12.8 (3.2)	12.5 (3.2)	11.8 (3.2)	12.1 (3.2)	11.0 (3.2)	< 0.001
Blood sampling variables						
Fasting at time of blood withdrawal, yes%	97.8	94.1	95.7	95.6	94.3	0.01
Blood sample collection area						
Amsterdam%	40.2	41.2	30.3	49.3	37.8	< 0.001
Leiden%	27.6	26.2	39.1	29.0	37.8	
Groningen%	31.2	32.6	30.6	21.7	24.4	
Metabolic assessment wave						
First%	62.5	24.9	93.4	33.2	94.7	< .001
Second%	37.5	75.1	6.6	66.8	5.3	
Lifestyle and somatic health						
Smoking status						
Current%	26.5	37.9	39.1	42.2	50.7	< 0.001
Former%	36.9	37.3	33.2	33.6	23.3	
Never%	36.6	24.7	27.6	24.3	26.1	
Number of glasses of alcohol per week, median (IQR)	3.7 (7.7)	3.7 (7.9)	2.4 (8.1)	3.7 (8.5)	2.4 (8.5)	< 0.001
Total MET-minutes per week, median (IQR)	3231.0 (3279.8)	3256.0 (3477.8)	2797.5 (3362.5)	3140.25 (3572.3)	2677.5 (3323.3)	< 0.001
BMI, median (IQR)	24.2 (5.7)	24.7 (5.8)	25.3 (6.7)	24.1 (5.9)	25.43 (6.9)	< 0.001
Number of chronic somatic diseases, median (IQR)	0.0 (1.0)	1.0 (1.0)	1.0 (1.0)	1.0 (1.0)	1.0 (2.0)	< 0.001
Diabetes Mellitus, yes%	4.1	3.2	3.9	4.0	5.1	0.54
Heart disease, yes%	6.9	6.9	7.9	9.7	11.5	0.02
Use of statins, yes%	6.3	6.0	6.6	7.3	8.5	0.44
Use of N-3 supplements, yes%	3.5	4.6	5.3	2.7	4.2	0.31
Clinical characteristics of depression and anxiety						
IDS-SR_{30} total score, mean (SD)	8.5 (7.5)	16.2 (9.6)	31.8 (10.5)	23.8 (10.2)	37.8 (10.8)	< 0.001
BAI total score, median (IQR)	4.0 (4.9)	8.1 (6.9)	14.9 (9.6)	16.1 (9.9)	23.0 (10.8)	< 0.001
FQ total score, median (IQR)	9.0 (14.25)	16.0 (18.15)	22.5 (24.0)	32.0 (25.0)	41.0 (29.0)	< 0.001
Antidepressant use						
No%	99.1	79.4	62.2	68.8	53.3	< 0.001
TCA%	0.2	2.5	2.6	4.6	4.3	
SSRI%	0.6	14.9	24.7	19.5	32.3	
Other antidepressant%	0.2	3.2	10.5	7.1	9.8	
Age of onset of first affective episode, mean (SD)	N.A.	N.A.	25.2 (12.8)	20.1 (11.9)	19.3 (12.3)	N.A.
Duration of affective symptoms (% of time), mean (SD)	N.A.	N.A.	34.2 (26.2)	39.6 (29.9)	52.2 (28.7)	N.A.
Type of depressive disorder						
MDD%	N.A.	N.A.	79.9	N.A.	59.7	N.A.
Dysthymia%			4.3		7.0	
Comorbid MDD and dysthymia %			15.8		33.3	
Major Depressive Disorder subtypes						
Severe atypical%	N.A.	N.A.	22.5	N.A.	28.4	N.A.
Severe melancholic%			41.0		52.4	
Moderate%			36.5		19.2	
Anxiety disorder subtypes						
Social phobia%	N.A.	N.A.	N.A.	24.6	14.7	N.A.
Panic disorder with agoraphobia%				16.4	10.6	
Panic disorder without agoraphobia%				8.6	6.0	
Agoraphobia%				9.9	19.1	
Generalized anxiety disorder%				28.3	45.9	

Note. Total n = 2912. IQR: Interquartile Range. MET: Metabolic Equivalent of Task. BMI: Body Mass Index. N-3: omega-3. IDS-SR_{30}: Inventory of Depressive Symptomatology − Self report 30 items. BAI: Beck's Anxiety Inventory. FQ: Fear Questionnaire. TCA: Tricyclic Antidepressant. SSRI: Selective Serotonin Reuptake Inhibitor. N.A.: Not Applicable. MDD: Major Depressive Disorder.

3. Results

3.1. Sample characteristics

The included 2912 participants had a mean age of 41.9 years (SD = 13.04, range 18–65) and 1935 (66.4%) participants were female (Table 1). In the total sample the median DHA level was 0.13 mmol/l (Interquartile Range (IQR) = 0.07), the median N-3 PUFA level was 0.36 mmol/l (IQR = 0.15) and the mean N-6 PUFA level was 3.94 mmol/l (SD = 0.75). The mean N-6:N-3 PUFA ratio was 10.94 (SD = 2.70). The median DHA:FA ratio was 1.13% (IQR = 0.49), the median N-3:FA ratio was 3.17% (IQR = 0.94) and the median N-6:FA

Table 2
Adjusted mean differences in circulating N-3 and N-6 PUFA levels (mmol/l) and N-3:FA ratio and N-6:FA ratio across disorder status groups.

	Controls (n = 634)		Remitted depressive/anxiety disorder (n = 897)		Current pure depressive disorder (n = 304)		Current pure Anxiety disorder (n = 548)		Current comorbid depressive and anxiety disorder (n = 529)		Overall *p*-value
	Adjusted mean	95% CI	Adjusted mean	95% CI	Adjusted mean	95% CI	Adjusted mean	95% CI	Adjusted mean	95% CI	
N-3 PUFA (mmol/l)[1]											
Model 1[2]	0.369[a]	0.358 – 0.381	0.360	0.351 – 0.371	0.355	0.341 – 0.369	0.359	0.348 – 0.371	0.350[a]	0.338 – 0.361	0.025
Model 2[3]	0.380[a]	0.362 – 0.398	0.370	0.354 – 0.387	0.364	0.345 – 0.384	0.371	0.353 – 0.389	0.360[a]	0.342 – 0.378	0.023
N-3:FA ratio (%)[1]											
Model 1[2]	3.30[ab]	3.22 – 3.39	3.21	3.14 – 3.29	3.13[b]	3.03 – 3.23	3.23[c]	3.15 – 3.31	3.10[ac]	3.01 – 3.18	0.009
Model 2[3]	3.64[ab]	3.51 – 3.78	3.57	3.45 – 3.70	3.47[b]	3.33 – 3.62	3.60	3.47 – 3.73	3.46[a]	3.33 – 3.60	0.001
N-6 PUFA (mmol/l)											
Model 1[2]	3.90	3.82 – 3.98	3.93	3.86 – 4.00	3.94	3.84 – 4.04	3.90	3.81 – 4.00	3.89	3.80 – 3.97	0.770
Model 2[3]	3.53	3.41 – 3.65	3.52	3.40 – 3.64	3.55	3.41 – 3.68	3.50	3.38 – 3.62	3.50	3.37 – 3.62	0.919
N-6:FA ratio (%)[1]											
Model 1[2]	34.1	33.7–34.5	34.5	34.1–34.8	34.1	33.7–34.6	34.1	33.7–34.5	34.1	33.7–34.5	0.613
Model 2[3]	33.3	32.8–33.8	33.5	33.0 – 34.0	33.3	32.8–33.9	33.4	32.9–34.0	33.4	32.9–34.0	0.917

Note. [1] Means and Confidence Intervals (CI) of transformed variables (according to natural logarithm) are back-transformed.[2] Model 1: Model corrected for sociodemographic and blood sampling variables: gender, age, education in years, fasting status at blood withdrawal, area of blood collection and number of metabolic assessment wave. [3] Model 2: additionally corrected for lifestyle variables and somatic health: alcohol, smoking, physical activity, diabetes, heart disease, number of chronic somatic disorder (except for diabetes and heart disease), Body Mass Index, statin use and use of N-3 PUFA supplements.

[a] Significant post hoc comparisons using Šidák correction between controls and current comorbid depressive and anxiety disorder with a significance level of < 0.05.

[b] Significant post hoc comparisons using Šidák correction between controls and current pure depressive disorder with a significance level of < 0.05.

[c] Significant post hoc comparisons using Šidák correction between current pure anxiety disorder and current comorbid depressive and anxiety disorder with a significance level of < 0.05.

ratio was 35.0% (IQR = 4.25). Supplementary Table 1 shows Spearman correlations between each PUFA measure, ranging from 0.01 (between N-6:FA and N-6 PUFA) to 0.90 (between N-3 PUFA and DHA). This indicates that measures are partly overlapping but not entirely similar, indicating that the secondary outcomes can give additional information next to the primary outcomes.

3.2. PUFAs and disorder diagnosis

Table 2 shows the adjusted means for the diagnosis groups and the overall *p*-value of the ANCOVA testing for significant differences in PUFAs levels across the overall psychiatric disorder status groups. Results for model 1 and 2 were significant after FDR correction for multiple testing with a q-value below 0.05 for N-3 PUFA and N-3:FA. In adjusted analyses, subsequent pairwise group comparisons for these PUFA markers showed that compared to controls, patients with current comorbid depressive and anxiety disorder had significantly lower N-3 PUFA levels (Model 2, Cohen's d = 0.09, p = 0.012). The N-3:FA ratio was significantly lower in patients with current comorbid depressive and anxiety disorder, and in patients with current pure depressive disorder compared to controls (Model 2, Cohen's d = 0.11, p = 0.002 and p = 0.021, Cohen's d = 0.11, respectively). These findings are graphically illustrated in supplementary Fig. 1.

3.3. PUFA's and depression and anxiety symptom severity

Fig. 1 shows the significant associations of N-3 PUFA levels and N-3:FA ratios with depression severity (IDS score, Beta = −0.042, p = 0.023 and Beta = −0.063, p = 0.001, respectively) and the significant association between the N-3:FA ratio and fear severity (FQ score, Beta = −0.037, p = 0.035) after FDR correction for multiple testing with a q-value below 0.05. Although in unadjusted models a significant association of N-6 PUFA levels with IDS was found (data not shown), in fully adjusted models, neither the IDS, BAI nor the FQ showed significant associations with N-6 PUFA levels or the N-6:FA ratios (Fig. 2).

3.4. PUFAs and clinical characteristics

Within patients, lower N-3:FA ratios were significantly associated with higher IDS scores (Table 3). N-3:FA ratio was also significantly lower in SSRI and other antidepressant users compared to non-users. Significantly lower N-3:FA ratios were found in comorbid MDD and dysthymia as compared to MDD. The N-6:FA ratio was borderline significantly lower in TCA and other antidepressants users compared to non-users. The N-6:FA ratio was significantly highest in social phobia, followed by the GAD, agoraphobia, and lowest in panic disorder (Table 3). Exploratory analysis showed that these subtypes do not have a significantly higher anxiety symptom severity (data not shown), so this could not be an explanation.

3.5. Post-hoc analysis with other PUFA measures and disorder status groups

Higher N-6:N-3 PUFA ratios were found in current comorbid depressive and anxiety disorder patients and current pure depressive disorder patients compared to controls (Cohen's d = −0.11, p = 0.001 and p = 0.015, Cohen's d = −0.11, respectively) (Supplementary Table 2). These effect sizes were comparable to those found for N-3 PUFA levels indicating that this result reflects the reported difference in N-3 PUFA levels across disorder status groups. Analyses showed significantly lower DHA levels in patients with comorbid depressive and anxiety disorder and patients with current pure depressive disorder compared to controls (p = 0.002, Cohen's d = 0.11 and p = 0.045, Cohen's d = 0.10, respectively). The DHA:FA ratio was significantly lower in patients with current comorbid depressive and anxiety disorder and patients with current pure depressive disorder compared to controls (p = 0.0002, Cohen's d = 0.12 and p = 0.003, Cohen's d = 0.13, respectively). This supports the N-3 PUFA findings, as DHA is a constituent of N-3 PUFA and therefore strongly correlated with the N-3 PUFA level (r = 0.90, p < 0.001). Results remained significant after FDR correction for multiple testing with a q-value below 0.05.

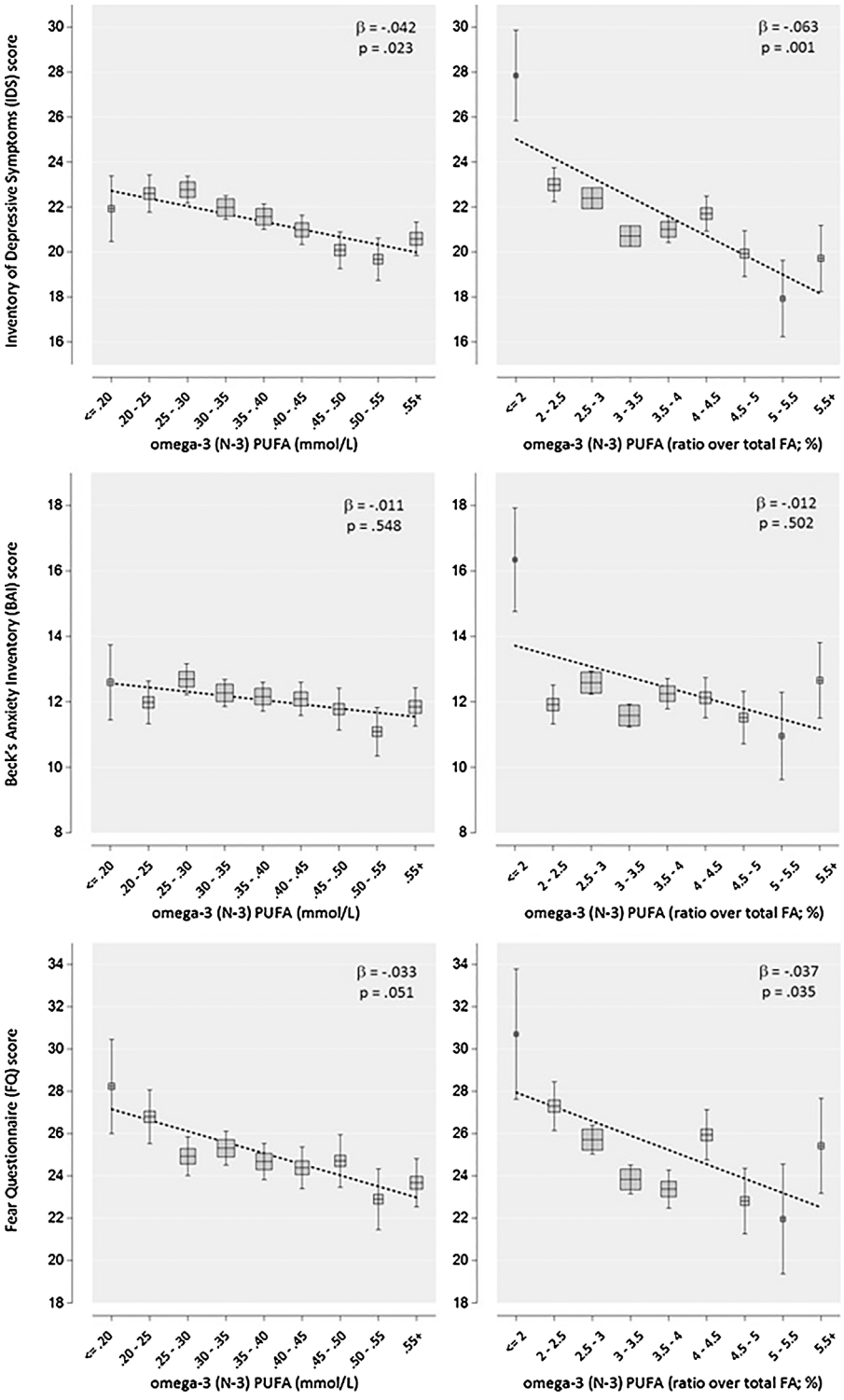

Fig. 1. Fully adjusted associations between untransformed N-3 PUFA levels and N-3:FA ratio and a) IDS score, b) BAI score and c) FQ score in the overall sample.
Note. β: standardized Beta. P: p-value. The size of each square is proportional to the number of participants. Vertical lines indicate standard errors. The dotted line represents the univariate regression line and beta-coefficients and p-values are calculated by linear regression analysis.

4. Discussion

We examined the relationship of circulating N-3 and N-6 PUFA levels with current and remitted depressive and anxiety disorders and several of their clinical characteristics. Results showed that lower N-3 PUFA levels are only observed in patients with a current depressive disorder, especially in the more severe group with comorbid anxiety. Furthermore, lower N-3 PUFA levels were associated in a dose-response fashion with higher depressive symptoms severity. Finally, within patients with current disorders, several clinical characteristics (e.g. depressive symptoms severity, antidepressant use and social phobia) were significantly associated with a lower N-3:FA or higher N-6:FA ratio. No specific pattern of association was detected for absolute N-6 PUFA levels.

Our findings indicate that the lower circulating N-3 PUFA levels in depressed patients are state-dependent, and therefore linked to a current episode, suggesting that low N-3 PUFA levels may not represent an underlying predisposing trait or a biological scar from previous episodes. Instead, N-3 PUFA alterations present in current patients may be reversible after remission, even within 1 month (the time interval we

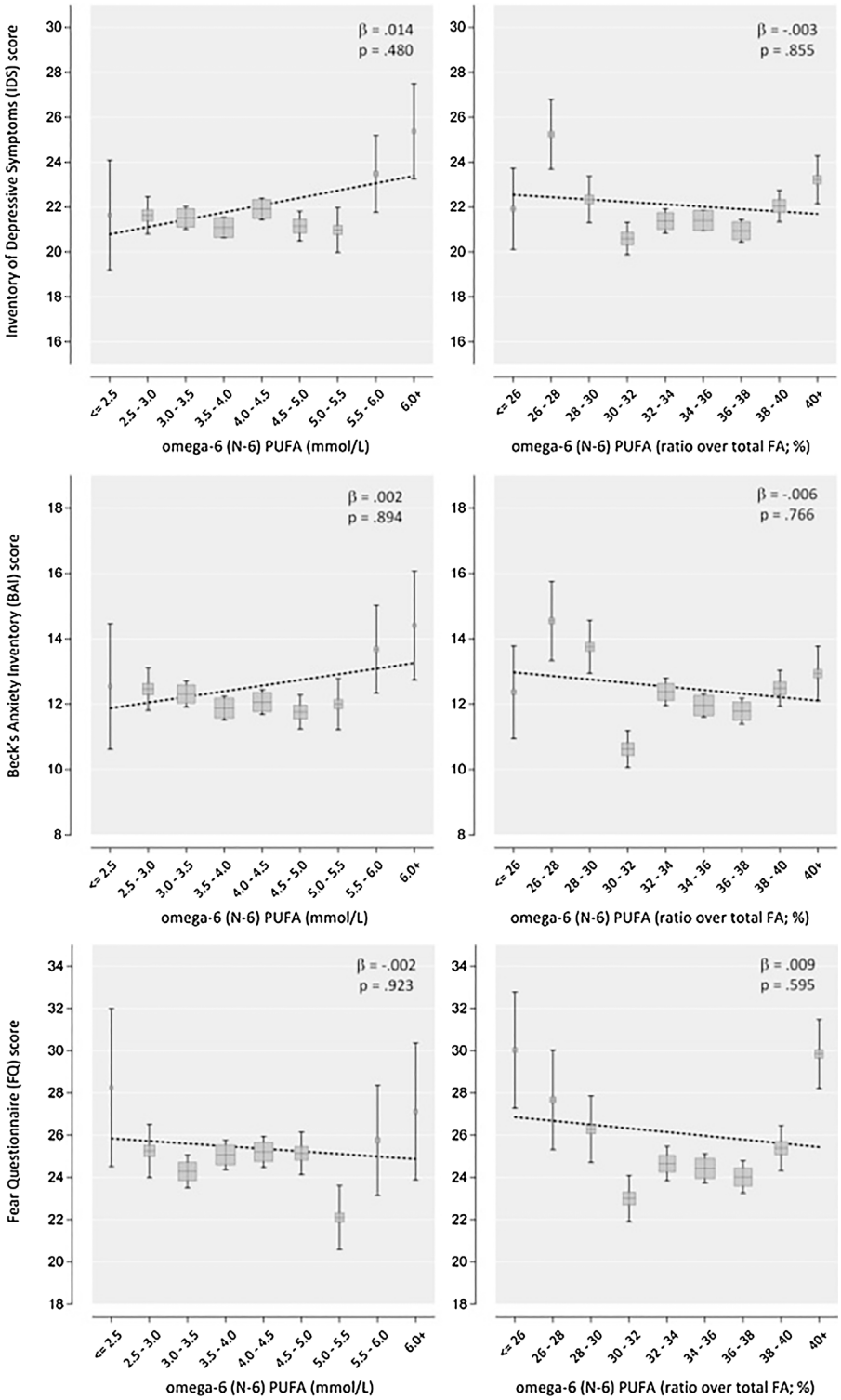

Fig. 2. Fully adjusted associations between untransformed N-6 PUFA levels and N-6:FA ratio and a) IDS score, b) BAI score and c) FQ score in the overall sample.
Note. β: standardized Beta. P: p-value. The size of each square is proportional to the number of participants. Vertical lines indicate standard errors. The dotted line represents the univariate regression line and beta-coefficients and p-values are calculated by linear regression analysis.

used to define remission). Although the effect size is small, it is of comparable magnitude to that of other biomarkers linked to the presence of depressive and anxiety disorders in the same cohort, such as inflammation markers (C-reactive protein and Interleukin-6) (Vogelzangs et al., 2012), brain-derived neurotrophic factor (Molendijk et al., 2011),and vitamin D (Milaneschi et al., 2014).

Our findings indicate that alterations in N-3 PUFA levels are specifically associated with depressive symptoms and disorders, and not with pure anxiety. The association between N-3 PUFA and depression is consistent with earlier research (Jacka et al., 2012; Lin et al., 2010; Liu et al., 2013; Smith et al., 2011; Wani et al., 2015). Associations between N-3 PUFA levels and N-3:FA ratio's and anxiety symptoms severity do not remain significant after adjustment, except for the association between N-3:FA ratio and phobic avoidance. In addition, N-3 PUFA levels do not differ between patients with current pure anxiety and healthy controls. The lowest circulating levels of N-3 PUFA are reported in current comorbid depression and anxiety, which is in line with a previous study (Liu et al., 2013). In this group comorbid anxiety may however be considered merely a marker of severity of depressive symptomatology, as indicated indeed by the highest IDS score reported

Table 3
Full adjusted associations between clinical characteristics and N-3 PUFA levels (mmol/l), N-3:FA ratio, N-6 PUFA levels (mmol/l) and N-6:FA ratio in patients with current disorders.

	LN N-3 PUFA[1] (mmol/l)		LN N-3 PUFA of total FA (%)[1]		N-6 PUFA (mmol/l)		LN N-6 PUFA of total FA (%)[1]	
	Beta[2]	*p*	Beta [2]	*p*	Beta [2]	*p*	Beta [2]	*p*
Current patient sample (n = 1381)								
IDS-SR_{30} per SD increase	−0.006	0.819	−0.057	0.048	0.047	0.107	−0.046	0.111
BAI per SD increase	0.025	0.311	0.020	0.440	0.009	0.735	−0.025	0.324
FQ per SD increase	−0.006	0.813	−0.021	0.405	0.011	0.667	−0.013	0.594
Antidepressant use[3]								
No	Ref.		Ref.		Ref.		Ref.	
TCA	0.003	0.902	−0.048	0.054	0.024	0.344	−0.074	0.003
SSRI	−0.004	0.855	−0.054	0.033	0.049	0.059	−0.038	0.130
Other antidepressants	0.005	0.830	-0.054	0.031	0.059	0.021	−0.053	0.035
Age of onset first affective episode per 10 years	0.006	0.823	0.001	0.983	−0.013	0.620	−0.051	0.053
Duration of symptoms (% of time)	0.030	0.211	0.018	0.452	0.037	0.142	0.011	0.659
Current depressive disorder sample (n = 833)								
Type of depressive disorder								
MDD	Ref.		Ref.		Ref.		Ref.	
Dysthymia	0.042	0.179	0.029	0.376	0.057	0.081	0.022	0.492
Comorbid MDD and dysthymia	−0.041	0.195	−0.070	0.031	−0.005	0.874	−0.044	0.176
Major depressive disorder subtypes								
Severe atypical	Ref.		Ref.		Ref.		Ref.	
Severe melancholic	−0.053	0.207	−0.060	0.171	−0.047	0.291	−0.070	0.105
Moderate	0.004	0.932	0.028	0.514	−0.043	0.323	−0.018	0.662
Current anxiety disorder sample (n = 1077)								
Anxiety disorder subtypes								
Social phobia	Ref.		Ref.		Ref.		Ref.	
Panic disorder with or without agoraphobia	−0.006	0.829	−0.019	0.532	−0.019	0.554	−0.080	0.010
Agoraphobia	0.030	0.308	−0.016	0.603	0.039	0.219	−0.073	0.018
Generalized anxiety disorder	0.004	0.882	−0.034	0.248	0.044	0.145	−0.030	0.304

Note. N = 1381. TCA: Tricyclic Antidepressant. SSRI: Selective Serotonin Reuptake Inhibitor. BAI: Becks Anxiety Inventory. FQ: Fear Questionnaire. IDS-SR_{30}: Inventory of depressive symptomatology − Self Report 30 items. MDD: Major Depressive Disorder. [1] Transformed according to natural logarithm. [2] Adjusted for sociodemographic variables, sample characteristics, lifestyle and somatic health.

by these patients. Jacka et al. (2012) did not adjust the significantly lower DHA intake in anxiety patients for the presence of comorbid depression, and were therefore not able to exclude comorbid depression as a cause of low DHA intake.

Within current patients, none of the clinical characteristics of depression or anxiety (e.g. severity, subtype age of onset, duration or antidepressant use) were associated with absolute N-3 or N-6 PUFA levels, which is not in line with previous studies (Bloch and Hannestad, 2012; Grosso et al., 2014). However, we found that lower N-3:FA ratios are associated with higher depression severity, and are lower in antidepressant users and in comorbid major depressive disorders and dysthymia. This is in line with our hypothesis of unfavorable PUFA levels in patients with a worse disease course and might be explained by the higher severity of depression that is found among antidepressant users and in comorbidity (Appleton et al., 2015; Bloch and Hannestad, 2012; Grosso et al., 2014). This means that severity of depression may be of help in identifying a subgroup of current patients at high risk for low N-3 PUFA levels to be specifically targeted in future supplementation studies. Against expectations and previous studies, we found lower N-6:FA ratios in antidepressant users compared to non-users and the highest ratio's in social phobia. A previous study on the same data showed that disability on multiple domains was generally highest in social phobia (Hendriks et al., 2014), suggesting that N-6:FA ratios may be higher in those with a higher anxiety disease burden.

No significant association is found with absolute N-6 PUFA levels. This is in line with a recent review (Smith et al., 2011), and suggests that not N-6 but merely N-3 PUFA plays a role in depression. However, N-3 and N-6 PUFA compete for the same desaturase enzymes for the conversion into other PUFAs. An increase in the N-6:N-3 PUFA ratio as observed in depressed patients, although driven by a reduction in N-3 PUFA, may determine an increased metabolic access of N-6 PUFA, which has been shown to be associated with several chronic diseases (Patterson et al., 2012).

Results indicate that most findings for absolute and relative PUFA values are comparable, except for the analysis with clinical characteristics: these were not associated with the absolute values. Relative values may be more informative than absolute values and in clinical practice both should be assessed. As results for DHA levels and the N-6:N-3 PUFA ratio were comparable to the results for N-3 PUFA levels, these measures seem to reflect similar constructs and can be used as additions to N-3 and N-6 PUFA levels. The reported high correlation between N-3 PUFA and DHA in our study (spearman's rho 0.90) is comparable to that in the study of Welch et al. (2006) also measured in phospholipids (spearman's rho 0.97).

There are several possible explanations for the relationship between N-3 PUFA levels and depression, one of them being lifestyle and somatic health factors. As N-3 PUFA intake is one of the strongest determinants of N-3 PUFA blood levels, there might be differences in fatty fish intake between healthy and depressed persons (Jacka et al., 2012). Fish meal preparation is more elaborate than other food items, which may be harder for depressed patients. Also, both depression and lower N-3 PUFA levels have been associated with smoking (Scaglia et al., 2016; Stepankova et al., 2016), alcohol use (Neupane, 2016; Pawlosky and Salem, 2004) and chronic somatic diseases (Giudetti and Cagnazzo, 2012; La Rovere and Christensen, 2015; Penninx et al., 2013; Simopoulos, 1999). Although we adjusted analyses for several lifestyle and somatic health variables, these adjustments did not have a significant impact on the observed associations. Furthermore, there are a number of potential biochemical mechanisms why low N-3 PUFA levels may influence depression pathophysiology: N-3 PUFAs exert anti-inflammatory actions, N-3 PUFAs are regulators of gene transcription in the CNS and play a role in neural membrane fluidity, ion channel functioning and receptor binding, and it was shown that a decrease in dietary DHA relates to decreased cortical serotonin and dopamine (Alessandri et al., 2004; Owen et al., 2008; Stahl et al., 2008; Young and Conquer, 2005).

One limitation is that no conclusion about the direction of the association between PUFA levels and depression can be made.

Unfortunately, we had no information on diet. Besides DHA, no other constituent of N-3 PUFA was measured, while a meta-analysis showed that especially EPA is effective in the treatment of depression (Mocking et al., 2016). However, given the high correlation between DHA and EPA when measured in phospholipids (Welch et al., 2006), it is to be expected that in the present study the current depressed patients also have lower EPA blood levels compared to healthy controls. Although NMR has a lower sensitivity than other methods, in other fields of research it has shown to be a reliable quantification (inter-assay coefficients of variation ranging from 2.7 to 4.5%) (Würtz et al., 2017). Important strengths of the present study were the large sample size (larger than the total sample size of a recent meta-analysis of observational studies on PUFA blood levels and depression (Lin et al., 2010)), the clinical characteristics based on well-validated questionnaires, the largely available blood samples, and the inclusion of both depression and anxiety patients and also both remitted and current patients.

5. Conclusions

In summary, it can be concluded that currently depressed patients (especially the more severe cases with comorbid anxiety), but not those with remitted disorder or a current pure anxiety disorder, have lower circulating N-3 PUFA levels than healthy controls. The results implicate that clinicians, practitioners and researchers should be aware that currently depressed patients (especially those with more severe symptomatology) may be at high risk for low N-3 PUFA levels. Further studies are needed to establish whether this subgroup would benefit from dietary supplementation. In addition, our findings suggest that not N-6 PUFA levels but merely N-3 PUFA levels play a role in depression. One should keep in mind that reported effect sizes were small. Future work using longitudinal data, properly designed trials and mechanistic studies are needed to fully elucidate the pathway linking N-3 PUFA levels with depression. Also, since N-3 PUFAs have been shown to have a potential impact on pathophysiological processes and chronic diseases (Giudetti and Cagnazzo, 2012; La Rovere and Christensen, 2015; Patterson et al., 2012; Simopoulos, 2002, 1999), their alteration may represent an important underlying mechanism for the somatic health outcomes of depression that cause a burden on functioning for patients (Penninx et al., 2013).

Conflict of interest

None of the authors have any conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.10.005.

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