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Polyunsaturated Fatty Acids Moderate the Effect of Poor Sleep on Depression Risk

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Abstract

Although potentially modifiable risk factors for interferon-alpha (IFN-α)-associated depression (IFN-MDD) have been identified, it is not currently known how they interact to confer risk. In the present study we prospectively investigated interactions among poor sleep quality, high stress, pre-existing depressive symptoms, and polyunsaturated fatty acid status. Non-depressed hepatitis C patients (n=104) were followed prospectively during IFN-α therapy. IFN-MDD occurs in 20-40% of patients and was diagnosed using the Structured Clinical Interview of DSM-IV (SCID-IV), with incidence examined using Cox regression. Baseline Pittsburgh Sleep Quality Inventory (PSQI), Perceived Stress Scale (PSS), Beck Depression Inventory (BDI), and a range of plasma long-chain fatty acid levels were measured (gas chromatography) -- focusing on the ratio of arachidonic acid (AA) to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (AA/EPA+DHA). The AA/EPA+DHA ratio (B=0.40±0.16; p=0.006), PSQI (B=0.12±0.04; p=0.001), PSS (B=0.07±0.02; p<0.001), and baseline BDI (B=0.05±0.02; p<0.001) each individually predicted IFN-MDD incidence. In step-wise Cox regression eliminating non-significant variables, two interactions remained significantly predictive: PSQI*AA/EPA+DHA (p=0.008) and PSS*AA/EPA+DHA (p=0.01). Receiver Operator Curves (ROC) were used to examine the specificity and sensitivity of IFN-MDD prediction. When sleep was normal (PSQI<5), AA/EPA+DHA was strongly predictive of IFN-MDD (AUC = 91 +/− 6; p=0.002). For example, among those with AA/EPA+DHA less than the median (4.15), none with PSQI<5 developed depression. Conversely, neither PSS nor PSQI was statistically associated with depression risk in those with an elevated AA/EPA+DHA ratio. These data demonstrate that the AA/EPA+DHA ratio moderates the effect of poor sleep on risk for developing IFN-MDD and may have broader implications for predicting and preventing MDD associated with inflammation.

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Keywords

Polyunsaturated fatty acids; Docosahexaenoic acid; Eicosapentaenoic acid; Arachidonic acid; Interferon-alpha; Sleep quality; Stress; Major depressive disorder

1. INTRODUCTION

Emerging evidence suggests that the pathophysiology of major depressive disorder (MDD) is frequently associated with chronic low-grade inflammation [1]. This is supported in part by findings that some MDD patients exhibit elevated levels of prostaglandin E_2 [2] and pro-inflammatory cytokines such as interleukin-6 [3]. Moreover, many initially non-depressed subjects develop a depressive episode during chronic administration of the pro-inflammatory cytokine interferon-alpha (IFN-α) [4-6]. Because 25-40% of patients develop interferon-associated depression (IFN-MDD) with 2-4 months of treatment [7], prospective examination of patients treated with IFN-α represents a valuable opportunity to identify risk and resilience factors. To date several putative modifiable risk factors for IFN-MDD have been identified [8], and developing a better understanding of how these risk factors interact may inform novel preventative strategies for IFN-MDD specifically and inflammation-associated MDD more generally.

1.1 Sleep is a risk factor for MDD

Poor sleep quality is commonly associated with subsequent depression incidence [9-11], and also predicts IFN-MDD [12, 13]. In particular, a physiological predictor of IFN-MDD is lower early night delta power [14], which also predicts MDD recurrence [15-17]. Whether improving sleep quality – specifically improving delta power during sleep – can prevent depression remains to be determined.

1.2 Long Chain fatty acid status is a risk factor for MDD

Deficits in the long-chain omega-3 (LC*n*-3) fatty acids eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) are associated with MDD [18]. In particular, the ratio of the omega-6 arachidonic acid (AA, 20:*n*-6) to LC*n*-3 fatty acids (the AA/EPA+DHA ratio) is elevated in MDD patients [19-24]. Likewise, IFN-MDD is also associated with a high AA/EPA+DHA ratio [25]. In fact, LC*n*-3 supplements have recently been demonstrated to prevent IFN-MDD in some people [26]. On proposed mechanism is via decreased inflammation [27], though direct brain effects may be plausible [28]. Although LC*n*-3 fatty acid levels may also interact with poor sleep quality to moderate adverse effects cognition [29], how or whether they interact with sleep quality to effect depression risk is not known.

1.3 Pre-existing sub-syndromal depression symptoms are a risk factor for MDD

Third, a history of prior MDD episodes and/or current sub-syndromal symptoms may predispose to IFN-MDD [30]. Prophylactic selective serotonin reuptake inhibitors (SSRIs) can halve the incidence of IFN-MDD [31-34]. Unfortunately, SSRIs don't eliminate the IFN-MDD incidence, and may have side effects such as retinopathy [33] and worsened irritable

anger [35, 36]. Although LC*n*-3 fatty acids have antidepressant properties [37] and may augment SSRI antidepressant effects [38, 39] it is not known how or whether LC*n*-3 fatty acid biostatus and MDD history interact to modify IFN-MDD risk.

1.4 Stress is a risk factor for MDD

Fourth, psychosocial stressors are a significant predictor of depressive episodes [40]. Consistent with this, altered hypothalamic-pituitary-adrenal axis activity may predict IFN-MDD [41, 42]. Interestingly, a fatty acid synthase polymorphism moderated the effect of perceived stress on depression [43] suggesting a plausible interaction between AA/EPA +DHA and stress.

The present study thus sought to prospectively investigate the interactions between the AA/EPA+DHA ratio and other risk factors such as psychosocial stress and sleep quality on depression vulnerability. Our primary hypothesis was that these risk factors would interact in a synergistic manner to increase risk for developing subsequent IFN-MDD. Based on prior evidence linking pro-inflammatory processes with poor sleep quality [44, 45], stressful life events [46-48], and an elevated AA/EPA+DHA ratio [24, 49-51], these findings may have relevance for predicting and preventing inflammation-associated MDD more broadly.

2. Materials and Methods

2.1. Subjects

Non-depressed adult subjects (between ages 18-80) were examined for plasma fatty acid levels and completed a set of questionnaires prior to subsequent IFN-α therapy for HCV (n=104). Our primary interest is IFN-MDD incidence. These subjects partially overlapped with those in a prior study [25]. Exclusion criteria were active mood, anxiety, psychotic, or drug/alcohol use disorders within 6 months prior to starting IFN-α treatment – using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV); known neurologic disease; or taking corticosteroids, antidepressants, anticonvulsants, and/or antipsychotics (although they could be taking as-needed sleeping medications). All subjects started weekly injections of pegylated (PEG) IFN-α2 (PEG-IFN-α2a: 135 μg/week or PEG-IFN-α2b: 120 or 150 μg/week) augmented with oral ribavirin. This study was approved by the University of Pittsburgh Institutional Review Board.

2.2. Assessments

Prior to initiating IFN-α therapy, and monthly for four months after therapy was initiated, depression was assessed using the Beck Depression Inventory-II (BDI) and the Montgomery-Asberg Depression Rating Scale (MADRS) as previously described (Lotrich et al., 2009). IFN-MDD was diagnosed using either an abbreviated SCID-IV interview focused on depression and/or in any subject started on an antidepressant by their physician -- as previously described in more detail [25]. The Perceived Stress Scale (PSS) [52], the life events scale (LES) [53, 54], and the Pittsburgh Sleep Quality Inventory (PSQI)(Buysse et al., 1989) were administered at baseline. For the LES, we examined both total number of stressful life events in the past year, as well the total score after adjusting each event for impact (self-rated on a scale from −3 to +3).

2.3. Gas chromatography

Plasma from whole blood was obtained from all subjects between 10AM and 4PM prior to initiating treatment for hepatitis C (HCV), and stored at −80°C until analysis. Folch reagent (2 mL Chloroform/Methanol 2:1) was added to 0.3ml of plasma to extract the lipid layer, dried under nitrogen, and reconstituted with chloroform (100 uL). The lipid extract was then transferred to a reversed-phase packed SPE column (Alltech, Nicholasville, KY) and washed with chloroform (10 mL), to remove triglycerides, and then acetone (10mL) to remove the cholesteryl esters. Phospholipids were then eluted with methanol (20mL), and the combined methanol fractions evaporated. The sample was methylated using NaOH/MeOH (0.5 mL) and the derivatization was completed with BF_3/MeOH followed by heating for 15 minutes at 85°C. To ensure total fatty acid methyl ester (FAME) extraction, NaCl (0.3 mL) was used before extraction with hexane. Sodium sulfate was added to the hexane layer to remove water, and the organic phase decanted and evaporated using nitrogen. Samples were then reconstituted with hexane (0.5 mL) and analyzed.

FAME's were analyzed using an HP 6890/5973 gas chromatograph/mass selective detector (Agilent Technologies, Santa Clara, CA). The column used to separate FAME's was an Agilent DB-FFAP 15m × 0.1 mm with 0.1 um of film thickness. Helium was used as carrier gas at a flow rate of 17.6 ml/min and a constant pressure of 53.8 psi. The initial temperature was set at 160°C and increased after injection of 1 ul of sample to 240°C at a rate of 15°C per minute. Once the temperature of 240°C was reached, it was maintained for 6 minutes for a total run time of 14.33 minutes. The transfer line was maintained at 280°C and the filament at 70Ev for EI. The data were evaluated using a TIC for compound identification and SCAN mode to measure relative percent of each fatty acid. Fatty acid identification was based on retention times of authenticated FAME standards (GLC 473B) and controls (GLC 462 and GLC 463) to ensure reproducibility (NuCheck Prep, Elysian, MN). Data are expressed as weight percent of total fatty acid pool (mg fatty acid/100 mg fatty acids). We assessed the omega-6 fatty acids linoleic acid (LA; C18:2n-6), gamma-linolenic acid (GLA; C18:3n-6), dihomo-gamma-linolenic acid (DGLA; C20:3n-6), and arachidonic acid (AA; C20:4n-6), and the omega-3 fatty acids alpha-linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3), and docosahexaenoic acid (DHA; C22:6n-3).

2.5. Statistical analyses

All statistics employed SPSS 22.0. We first examined IFN-MDD incidence (i.e. days after starting IFN-α therapy until MDD developed), following patients for 120 days after starting IFN-α therapy. Potential reasons for censored data were that patients quit IFN-α therapy or IFN-α therapy was stopped prematurely because of medical side effects. Cox regression analyses were employed in four stages. First, we tested the assumption of proportional hazards using two techniques: **i**. Graphical inspection of log-hazard vs. time, and the log (−log) survival plot. **ii**. testing the interaction of time by study group.

Next, we examined individual candidate variables that are likely influences on risk for IFN-MDD, both without covariates and then including co-variates such as age and weight. Third, we examined hypothesized interactions, both with and without covariates. Finally, we then

included each variable (including interactions and covariates) into a step-wise Cox regression model, using conditional modeling to eliminate non-contributing variables.

All variables met the proportional hazards assumption except for AA/DHA+EPA, thus any interactions wit fatty acids found using Cox-regression were suspect. We therefore examined interactions with AA/DHA+EPA using several additional statistical approaches. First, we divided variables (AA/DHA+EPA, PSQI, and PSS) into quartiles. We utilized Kaplan-Meier with Mantel-Cox log rank comparisons to examine the AA/DHA+EPA quartiles, stratified by either PSQI or PSS. Secondly, we also used Receiver Operating Curve (ROC) analyses to examine sensitivity and specificity. ROC analyses examine predictive tests by plotting the false positive rate (1-specificity) against the true positive rate (sensitivity). The area under the curve (AUC) for this plot ranges from 100 (perfect performance) to 50 (uninformative) [55]. We examined the ability of baseline AA/DHA +EPA to predict subsequent IFN-MDD, when stratified by PSQI.

Finally, the categorical diagnosis of “MDD” is only one way of defining depression. To examine interactions using a quantitative measure and confirm any findings from the survival analyses, we analyzed MADRS scores over time (where day one was the first day of IFN-α therapy) using a mixed-effect repeated-measure analysis. We dichotomized the variables (AA/DHA+EPA, PSQI, and PSS) using a median split and test for interactions: AA/EPA+DHA*PSQI*time and AA/EPA+DHA*PSS*time.

3. Results

3.1 Subject characteristics

Subjects were 2/3 male, mostly Caucasian, often overweight, and 40% had a history of any mood disorder in remission (**Table 1**). Both age and female gender were associated with lower ratios of AA/EPA+DHA (**Table 2**). None of the long-chain fatty acids were associated with weight; but self-reported African-American heritage was associated with evidence of greater delta-5 desaturase activity (as manifest in an elevated AA/DGLA ratio) and higher levels of DHA+EPA, as consistent with prior reports [56]. Age was associated with higher DHA levels; and females had lower AA/DGLA ratios and higher ALA levels (**Table 2**). Because of these various relationships, in subsequent analyses we controlled for age, weight, gender, and race. Consistent with prior studies, the only fatty acid variable predictive of IFN-MDD development was AA/EPA+DHA (**Table 2**), whether controlling for covariates or not.

3.21 Depression incidence with Cox regression

In Cox-regression analyses of depression development, the AA/EPA+DHA ratio, PSQI, PSS, and BDI all predicted IFN-MDD incidence, when examined individually or when co-varying for race, weight, age, and gender (**Table 3**). When also controlling for baseline BDI, AA/EPA+DHA and PSQI both continued to predict IFN-MDD (p=0.002 for each). Actual stressful life events did not influence risk for IFN-MDD, but rather the subjective perception of stress (PSS) did (**Table 3**). However, when controlling for baseline BDI, PSS no longer was significant (p=0.13).

AA/EPA+DHA interacted with both PSQI and PSS to predict IFN-MDD when other covariates were included (**Table 3**). When additionally co-varying for baseline BDI, both of these interactions remained significant (P<0.001 and p=0.011, respectively). We next added each of these variables ***stepwise*** into a single multivariate Cox regression model – sequentially including both interactions (PSQI*AA/EPA+DHA and PSS*AA/EPA+DHA), as well as AA/EPA+DHA, BDI, PSQI, PSS, race, age, gender, and weight. Because most of these variables are correlated with the others (**Table 1**), conditional modeling was employed to eliminate non-contributing variables (both the Wald or Likelihood Ratio produced similar results). The only remaining three variables that predicted IFN-MDD incidence were PSQI*AA/EPA+DHA (B=0.029+/−0.008; p=0.008), PSS*AA/EPA+DHA (B=0.01+/0.004; p=0.01) and race (B=1.038 +/− 0.008; p=0.04).

Because AA/EPA+DHA and PSQI are correlated at baseline (**Table 1**), we also examined if one might mediate the other's effects. When co-entered into Cox-regression analyses of IFN-MDD incidence (along with weight, age, gender, race), both continued to be significant (B=0.57+/−0.18; p=0.002 and B=0.12+/−0.04; p=0.007 respectively). Thus the influence of AA/EPA+DHA on depression risk is not because it is correlated with PSQI.

3.22 Depression incidence with Kaplan-Meier

Although the interactions between AA/EPA+DHA were robust, the assumption of proportional hazards was not met by AA/DHA+EPA. Therefore, we divided AA/EPA+DHA (as well as PSQI and PSS) into quartiles for analysis using log-rank comparisons. When PSQI was in the lowest quartile (PSQI<5), AA/EPA+DHA was strongly predictive of IFN-MDD (X^2 = 17.7; p=0.001). In fact, 100% of subjects with the highest quartile of AA/EPA +DHA (AA/EPA+DHA>4.95%) developed IFN-MDD while 0% of the subjects in the lower fatty acid quartile (AA/EPA+DHA<3.5%) did. Conversely, for the other three quartiles of PSQI, AA/EPA+DHA was no longer predictive at all (p>0.3). This supports the interaction between AA/EPA+DHA and PSQI noted above – where fatty acids are only predictive when PSQI is low. The interaction with stress was less evident. AA/EPA+DHA was no longer predictive for any quartile of PSS (p >0.13 for all four quartiles).

To explore potential clinical utility, we also combined z-scores for PSS, PSQI, and AA/DHA+EPA into a single measure for log-rank analysis. In Kaplan-Meier analyses, the lowest quartile of this combined z-score were almost all resilient whereas the highest quartile were almost all vulnerable to depression during IFN-α treatment (X^2 = 18.2; p<0.001) (**Fig. 1**).

3.3 Receiver Operating Curve (ROC) analyses of depression prediction

To better define the nature of the significant interactions, we split AA/EPA+DHA ratios into greater or less than the median (median=4.15, range 1.47 to 7.06); and then examined the predictive utility of baseline BDI, PSS, and PSQI using ROC analyses. When AA/EPA +DHA was <4.15, the ROC AUC for PSQI was 76 +/− 9 (p=0.02). In fact for all subjects with PSQI<5 and AA/EPA+DHA<4.15, sensitivity at predicting total resilience to IFN-MDD was 100%. However, PSQI was not predictive of IFN-MDD when AA/EPA +DHA>4.5 (p=0.21).

Baseline BDI was similarly predictive of IFN-MDD regardless of having high or low AA/EPA+DHA (AUC = 68 +/− 9; p=0.045 and 67 +/− 8; p=0.045). PSS lost power to predict subsequent IFN-MDD, whether AA/EPA+DHA was less than or greater than the median, (p=0.49 and 0=0.72, respectively). Thus the moderation of sleep quality's effects by AA/EPA+DHA was the only consistent interaction associated with IFN-MDD development.

We also examined the ability of AA/EPA+DHA to predict IFN-MDD, as moderated by PSQI. PSQI was split into tertiles (<5, 5-8, >8), and ROC curves were assessed for AA/EPA +DHA (**Figure 2**). When PSQI was low (<5), AA/EPA+DHA was strongly predictive of IFN-MDD (AUC = 91 +/− 6; p=0.002). However when PSQI was medium (5-8) or high (>8), AA/EPA+DHA was no longer predictive (AUC = 71 +/− 10 p=0.08 and AUC = 46+/− 11; p=0.8).

3.4 Repeated-measure mixed-effect analysis of symptom development

This interaction between sleep and fatty acids was confirmed in repeated-measure mixed-effect analyses of MADRS scores over time. For this, we examined median splits for PSS, PSQI, and AA/EPA+DHA to dichotomize each into ‘high’ and ‘low’ groups. We replicated the interaction between AA/EPA+DHA and time - those with greater AA/EPA+DHA have increasing MADRS scores after IFN-α is started ($F=3.9_{(9,42.9)}$; p=0.001). Consistent with the findings above, there was an interaction between PSQI*AA/EPA+DHA*time ($F=2.2_{(10,39.3)}$; p=0.042). However, there was no similar PSS*AA/EPA+DHA*time interaction (p>0.4).

4. Discussion

Self-reported sleep quality and LC*n*-3 status interact to affect resilience to inflammatory-cytokine-associated depression. This interaction indicates that LC*n*-3 status (i.e. AA/EPA +DHA) moderates the influence of sleep quality on depression risk. We have previously reported on the beneficial effect of good sleep [13], which is often reflected by PSQI <5 [57]. However, a number of patients still develop IFN-MDD despite sleeping well. We now report that in these good sleeping subjects, the AA/EPA+DHA ratio was highly influential on risk for IFN-MDD. The AUC for the receiver operating curve was >0.9 for AA/EPA +DHA, indicative of a test with excellent predictive performance [58]. However, in those sleeping poorly, the AA/EPA+DHA ratio lost much of its predictive power.

Our findings suggest that AA/EPA+DHA levels are less influential in those with poor sleep. In a recent placebo-controlled prevention trial, supplementation with EPA improved resilience, but 10% still developed IFN-MDD [26], indicating a significant amount of IFN-MDD risk remains even following supplementation. Our results predict that (i) optimal benefits of LC*n*-3 supplementation will be seen in those that are sleeping well, and (ii) optimal benefits of sleep interventions will occur in those with low AA/EPA+DHA ratios.

We also observed that PSQI scores correlated with AA/EPA+DHA at baseline, prior to any IFN-α injection. In fact, this correlation continued to be significant even when co-varying for both BDI and PSS. LC*n*-3 fatty acids may influence sleep quality [59-61]; and consistent with our observation, poor sleep correlated with the AA/DHA ratio in children [62] and with

lower EPA and DHA levels in depressed inpatients [63]. It is thus possible that fatty acid supplementation can also improve sleep [60, 64]. However, any causal link between fatty acids and sleep is speculative at this point. Of note, we did not find any evidence that sleep mediated the effects of AA/EPA+DHA on depression risk.

Unlike the interaction with sleep, our findings did not support any influence of AA/EPA +DHA on the risk related to baseline BDI. Treating pre-existing sub-syndromal depression symptoms may be important in preventing IFN-MDD [32, 65], but our results predict that these benefits of SSRIs do not depend on LC*n*-3 levels. Also, stress had limited predictive power. PSS scores were strongly correlated with BDI scores (R=0.7), and only somewhat correlated with stressful life events. This is consistent with observations that the subjective perception of stress is likely related to mood state and can improve with antidepressant treatment [66]. PSS lost the power to predict IFN-MDD when AA/EPA+DHA was used to split the population into subgroups; and LES had no predictive power. PSS also did not interact with AA/EPA+DHA in the repeated measures analysis of MADRS depression symptoms during IFN–α therapy. It is possible that we lacked statistical power to detect smaller effect sizes. Thus potential findings are only suggestive, and the role of stress in increasing vulnerability to inflammation requires further investigation. Nonetheless, patients with combined poor sleep quality, high perceived stress, and elevated AA/(EPA+DHA) are at highest risk of developing depression in response to IFN-α treatment.

Importantly in these analyses, we controlled for race, gender, weight, and age. Delta-5-desaturase and delta-6 desaturase are the rate-limiting enzymes for polyunsaturated fatty acid conversion, and are recognized as main determinants (other than diet) of PUFA levels. Genetic polymorphism studies [67] support our findings of higher delta-5-desaturase activity in African-Americans [68, 69]. We also observed evidence that females have higher delta-5-desaturase activity [70]. Interestingly African-Americans had higher EPA+DHA levels. This is despite the observation that African-Americans were about 4% more likely to develop IFN-MDD. There are likely other influences on risk for IFN-MDD that co-vary with racial background and gender [71, 72]. Thus, controlling for these variables is important in clinical studies of LC*n*-3 and depression.

One limitation of our study is that neither diet nor exercise was measured. Exercise can influence sleep quality [73], and diet is an obvious influence on fatty acid levels. A strength of this study is the prospective design and longitudinal assessment of IFN-MDD incidence. Another strength is the measurement of the ratio of AA/EPA+DHA, which may be more physiologically meaningful than straightforward LC*n*-3 levels [74-76]. This study did not address the physiological mechanism that might underlie the interactions. LC*n*-3 levels correlate with brain serotonin [77, 78] [79]; and with glutamate receptor subunit expression [80] [81, 82] [83]. Brain-derived neurotrophic factor (BDNF) genotype is associated with increased vulnerability to IFN-MDD [84], and LC*n*-3 fatty acids increase BDNF production [28, 85]. The effects of sleep on depression risk may also involve serotonin [86], glutamate [87], and growth factors [88, 89]. LC*n*-3 fatty acids may also blunt stress-induced elevations in inflammatory cytokines [27]. Thus, there are several mechanisms by which interactions are plausibly feasible.

Regardless of mechanism, the interaction between sleep and fatty acids on depression risk has important clinical implications. MDD is a leading cause of disability and suicide [90-92] and has adverse effects on the prognosis for those with co-morbid medical illness [93]. Targeted attention to modifiable, interacting risk factors like sleep and fatty acid levels may be critical for depression prevention. In brief, efforts to improve resiliency will be optimal if both sleep quality and AA/EPA+DHA are concomitantly addressed.

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Abbreviations

ALA	Alpha-linolenic acid
AA	Arachidonic acid
BDI	Beck Depression Inventory
DGLA	Dihomo-gamma-linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
GLA	Gamma-linolenic acid
IFN-α	Interferon-alpha
IFN-MDD	Interferon-alpha-associated depression
LES	Life events scale
LA	Linoleic acid
LC*n*-3	Long chain omega-3
MDD	Major depressive disorder
MADRS	Montgomery-Asberg Depression Rating Scale
PSS	Perceived Stress Scale
PSQI	Pittsburgh Sleep Quality Inventory
PUFA	Polyunsaturated fatty acid
ROC	Receiver operator curve
SSRI	Selective serotonin reuptake inhibitor
SCID-IV	Structured Clinical Interview of DSM-IV

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Highlights

- Sleep, the AA/EPA+DHA ratio, and stress each influence risk for depression
- Sleep and AA/EPA+DHA interact - the AA/EPA+DHA effect is strongest in good sleepers
- Conversely, the role of sleep is strongest in those with low AA/EPA+DHA ratios
- The role of baseline depression remained independent of AA/EPA+DHA ratios
- The role of stress was less robust, but may interact with AA/EPA+DHA

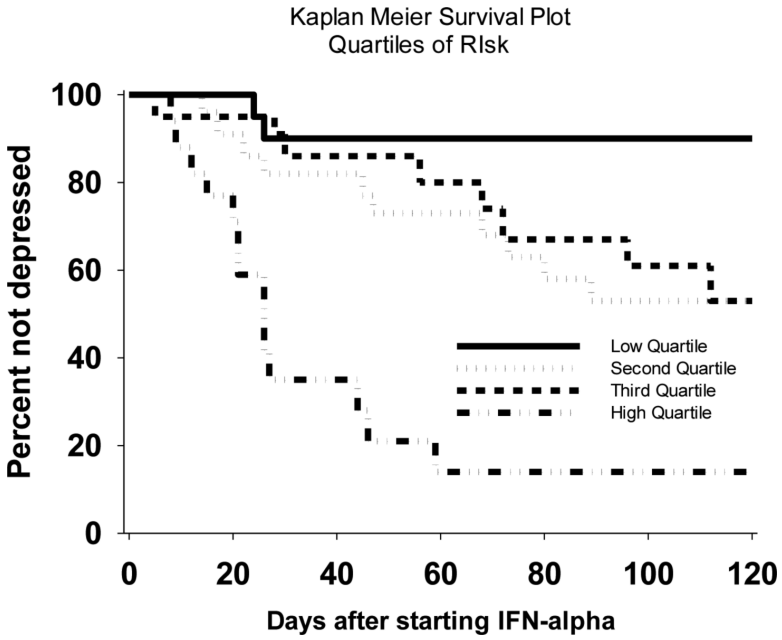

Figure 1.

Kaplan-Meier survival plot of the quartiles for combined Z-score for fatty acids, PSS, and PSQI. Few patients in the lower quartile developed depression whereas the majority of patients in the upper quartile developed depression.

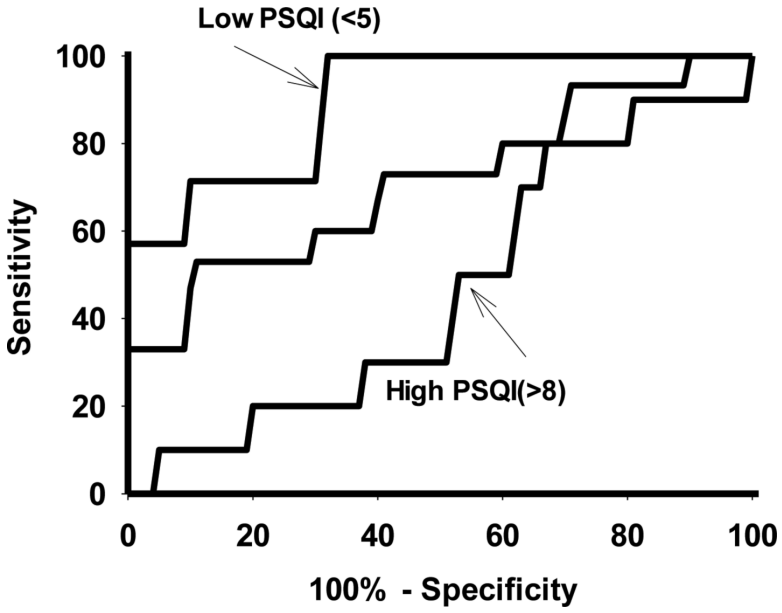

Figure2.

Receiver Operating Curves (ROC) for fatty acid ratio (AA/EPA+DHA). Three ROC curves were obtained: when Pittsburgh Sleep Quality Inventory tertiles of <5 (good sleep), 5-8 (mild sleep problems), and >8 (significant sleep problems).

Table 1

Demographic characteristics (mean +/− standard deviation) of the study population, and correlations with perceived stress (PSS), sleep quality (PSQI), and ratio of omega-6/omega3 fatty acids (AA/EPA+DHA). Significant ($p<0.05$) results for correlations (R) are indicated in bold and with an asterisk.

	Mean +/− SD	R with PSS	R with PSQI	R with AA/EPA+DHA
Female	33%	0.073	0.04	**0.27***
African-American	15%	0.10	0.03	0.07
Age	47.9 +/− 11	**0.20***	0.09	**0.27***
Weight (Kg)	86.6 +/− 17.6	0.09	0.05	0.01
CRP	2.1 +/− 2.6	0.05	0.02	0.01
MADRS	3.6 +/− 4.4	**0.38***	**0.36***	0.19
BDI	9./1 +/− 7.9	**0.70***	**0.52***	**0.26***
History of depression	40.0%	**0.37***	**0.25***	0.17
LES Total number	6.1 +/− 4.3	**0.31***	**0.28***	0.04
LES Total score	−2.5 +/− 8.7	**0.28***	**0.23***	0.20 (p=0.07)
PSS	19.6 +/− 8.6		**0.38***	0.12
PSQI	6.6 +/− 4.1			**0.31***
AA/EPA+DHA	4.2 +/− 1.1			

Table 2

Mean percent of total fatty acids (+/− standard deviations) for seven long chain fatty acids, and correlations (R values) with demographics are presented. Statistical significance ($p<0.05$), not correcting for multiple testing, is labeled in bold and with an asterisk. Only one of these (AA/EPA+DHA) predicted depression during interferon treatment (IFN-MDD).

Fatty Acids		**Percent of total**	**Correlations**				**Cox-Regression with IFN-MDD**
			Gender	**Race**	**Age**	**Weight**	
LA	C18:2*n*-6	23.6 +/− 2.8%	.02	.16	.06	.1	B = −0.08 +/− 0.06; N.S.
GLA	C18:3*n*-6	0.08 +/− 0.05%	.01	.17	.08	.1	B = −0.73 +/− 3.0; N.S.
DGLA	C20:3*n*-6	3.5 +/− 0.8%	.14	**.29***	.08	.09	B = 0.09 +/− 0.18; N.S.
AA	C20:4*n*-6	10.9 +/− 2.1%	.12	.17	.05	.11	B = 0.05 +/− 0.07; N.S.
ALA	C18:3*n*-3	0.19 +/− 0.08%	**.25***	.04	.04	.19	B = −0.13 +/− 1.9; N.S.
EPA	C20:5*n*-3	0.46 +/− 0.19%	.12	.03	.07	.08	B = 1.1 +/− 0.8; N.S.
DHA	C22:6*n*-3	2.3 +/− 0.8%	.14	**.24***	**.21***	.08	B = −0.3 +/− 0.2; N.S.
GLA/LA	Delta-6 desaturase index		.01	.15	.1	.09	B = 4.7 +/− 6.2; N.S.
DGLA/LA	Delta-6 desaturase index		.11	.18	.08	.12	B = 3.4 +/− 3.6; N.S.
AA/DGLA	Delta-5 desaturase index		**.22***	**.35***	.12	.03	B = −0.03 +/− 0.145; N.S.
EPA+DHA	Combined LC*n*-3		.15	**.22***	**.21***	.06	B = −0.23 +/− 0.18; N.S.
AA/EPA+DHA	Ratio of LC*n*-6/3.		**.27***	.07	**.27***	.01	B = **0.4 +/− 0.15; p=0.008**

Table 3

The fatty acid ratio (AA/EPA+DHA), perceived stress (PSS), sleep quality (PSQI), and baseline depression symptoms (BDI) all predicted IFN-MDD in Cox-regression survival analyses. Two measures of stressful life events did not. With covariates included, two interactions also predicted depression. The interaction between depression and fatty acids lost significance when covariates were included.

	Without covariates	**With covariates (age, weight, gender, race)**
AA/EPA+DHA	B=0.40±0.15; **p=0.008**	B=0.56+/−0.17; **p=0.001**
PSS	B=0.07±0.02; **p<0.001**	B=0.036 +/−0.014; **p=0.01**
PSQI	B=0.12±0.04; **p<0.002**	B=0.13+/−0.04; **p<0.001**
BDI	B=0.05±0.02; **p<0.001**	B=0.06+/−0.02; **p<0.001**
LES (total #)	B=0.06 +/−0.03; p=0.07	B=−0.02 +/−0.05; p=0.6
LES (total score)	B=0.0+/−0.02; p=0.9	B=0.03+/−0.02; p=0.12
PSS*AA/EPA+DHA	B=0.01+/−0.004; **p=0.006**	**B=0.009+/−0.004; p=0.035**
PSQI*AA/EPA+DHA	B=0.03+/−0.007; **p<0.001**	**B=0.023+/−0.009; p=0.012**
BDI*AA/EPA+DHA	B=0.019+/−0.005; **p<0.001**	B=0.011+/−0.007; p=0.13