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Eicosapentaenoic acid in serum lipids could be inversely correlated with severity of clinical symptomatology in Croatian war veterans with posttraumatic stress disorder

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Aim To explore the association between plasma fatty acids composition and the severity of clinical symptoms in Croatian war veterans with posttraumatic stress disorder (PTSD).

Methods This cross-sectional study included 62 men diagnosed with PTSD caused by combat activities during the War in Croatia 1991-1995. Clinician-Administered PTSD Scale (CAPS), Hamilton Anxiety Rating Scale (HAM-A), and Hamilton Depression Rating Scale (HAM-D-17) were used. Plasma fatty acids composition was determined by gas chromatography. Data about life-style habits were collected by a structured interview. To evaluate the association between plasma fatty acid levels and PTSD severity scales, multivariate general linear models (GLM) were applied while controlling for different confounders.

Results Significant negative correlations were found between plasma eicosapentaenoic acid (EPA, 20:5n-3) level and the scores on psychological scales ($\tau = -0.326$, $P < 0.001$ for CAPS; τ-0.304, $P = 0.001$ for HAM-A; and $\tau = -0.345$, $P < 0.001$ for HAM-D-17). GLM confirmed that PTSD severity was affected by EPA (Wilks'$\Lambda = 0.763$-0.805, $P = 0.006$-0.018, η_p 0.195-0.237), arachidonic acid (AA)/EPA (Wilks'$\Lambda = 0.699$-0.757, $P = 0.004$, η_p 0.243-0.301), and dairy products consumption (Wilks'$\Lambda = 0.760$-0.791, $P = 0.045$-0.088, η_p 0.128-0.111). No other fatty acid or dietary/lifestyle variable was significant ($P = 0.362$-0.633).

Conclusion The study suggests that lower EPA levels are associated with the severity of clinical symptoms in PTSD.

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Over the last decade, scientific community has developed a rapidly growing interest in the associations between metabolism of n-3 polyunsaturated fatty acids (PUFA) and a wide range of psychiatric disorders (1). Fish and marine derivatives, such as fish oil, are the primary sources of docosahexaenoic acid (DHA; C22:6n-3) and eicosapentaenoic acid (EPA; C20:5n-3), the two key n-3 PUFA present in brain lipids, particularly phospholipids. n-3 PUFA exhibit various physiological properties: neuroinflammatory, vascular, and gene control properties. They also play a significant role in the composition and fluidity of neuron membranes and consequently have regulatory effects on various neurotransmitter systems or signal transduction (2,3). Both EPA and DHA maintain properties of the lipid bilayer within cellular membranes (4). They concentrate at synapses in the human brain and are essential for neuronal functioning (5). Experiments with animals on fat-free diet showed that metabolic mechanisms tended to compensate for the lack of essential fatty acids by increased synthesis of endogenous unsaturated fatty acids (6). However, since n-6 and n-3 unsaturated fatty acids compete for the same metabolic pathways, the balance between n-6 and n-3 PUFA is important for the overall optimal functioning of the organism (7). n-6 and n-3 PUFA cannot be synthesized by the human body and must be supplied by nutritional sources. Therefore, the ratios of n-6/n-3 PUFA, arachidonic acid (C 20:4n-6) to EPA (AA/EPA) and AA/DHA can be used for nutritional assessment. Furthermore, AA and EPA are used as initial substrates in the synthesis of eicosanoids with pro- (ie, AA) or anti-inflammatory (ie, EPA) effects, and the AA/EPA ratio indicates which one might dominate. EPA and DHA are the main n-3 PUFA in the brain, but it is still not clear whether one of them (and which one) is more important, or if their ratio is a prerequisite for proper functioning of the brain.

Significant negative correlation has been demonstrated between annual fish consumption and the prevalence of major depression, as well as between the severity of depressive symptoms and plasma levels of n-3 PUFA (8,9). Higher n-6/n-3 ratio has been observed in depressed patients than in non-depressed controls, thus demonstrating the implication of n-6 PUFA in depression (8-10).

Psychological stress was found to be associated with altered proportions of n-6 unsaturated plasma fatty acids (11) and n-6/n-3 PUFA ratio was related to alterations in serotonergic, as well as in catecholaminergic neurotransmission system (5), whereas the n-3 PUFA deficit was related to the alteration in serotonergic and dopaminergic systems (12).

These neurotransmitter systems are involved in the neurobiological model of posttraumatic stress disorder (PTSD) (13). PTSD is an anxiety disorder caused by exposure to a traumatic event that involved a threat to the physical integrity of self or others (14).

PTSD symptoms are significantly alleviated in patients who took n-3 fatty acids after the injury causative to their PTSD (15). Also, recent case-control and n-3 PUFA supplementation clinical trials suggest that an increased intake of n-3 PUFA, whether through whole foods or supplements, may be beneficial in conditions of acute stress and some types of anxiety disorders, which points to possible abnormalities of fatty acids composition in anxiety disorders (7,16). The aim of our study was to test the associations between fatty acid levels and the severity of PTSD symptomatology. We hypothesized that low levels of EPA and DHA, or total n-3 PUFA, were associated with an increased severity of PTSD symptoms.

PARTICIPANTS AND METHODS

Participants

This cross-sectional study included patients from the pool of veterans involved in our outpatient PTSD program at the Department for General Psychiatry, University Psychiatric Hospital Vrapče, Zagreb, Croatia. The study was approved by the Ethics Committee of the University Psychiatric Hospital Vrapče, Zagreb, Croatia. Participants were consecutively recruited from June 2010 till February 2011, and all 62 included participants were male outpatient veterans. Patients were eligible if they met the following inclusion criteria: (i) met the DSM-IV-TR (14) and the International Classification of Disorder (ICD-10) (17) diagnostic criteria for PTSD, which was reestablished by experienced certified psychiatrists during the inclusion screening procedures; (ii) participated in the War in Croatia 1991-1995, (iii) had the Clinician-Administered PTSD Scale (CAPS) (18) score equal to or greater than 50 (19), and (iv) had been on a stable dose of sertraline (50 mg/d) for at least three months continuously prior to the study beginning. The exclusion criteria were as follows: (i) used n-3 PUFA supplements any time in the past, (ii) used any medication (other than sertraline) two weeks prior to the study beginning, (iii) had concurrent psychiatric or somatic disease, and (iv) suffered from alcohol or drug abuse. Participation in the study was voluntary and prior to any study procedure all participants signed an informed consent.

Antidepressants

The two antidepressant drugs, sertraline and paroxetine, have Food and Drug Administration's approval for the treatment of PTSD (20,21). It seems that antidepressants have no relevant effect on composition of fatty acids (22,23), but paroxetine can cause weight gain and, therefore, influence the body mass index (BMI) (21). As BMI appeared as a co-founder in some studies about the fatty acid profile and psychiatric symptomatology (23,24), we included only veterans who used sertraline alone.

Questionnaires

In this study four questionnaires were used: Clinician-Administered PTSD Scale (CAPS), Hamilton Anxiety Rating Scale (HAM-A) (25), Hamilton Depression Rating Scale-17 (HAM-D-17) (26), and a socio-demographic questionnaire (27). Experienced psychiatrists who administered the scales were trained and certified for the implementation of each specific scale in its original English version. Considering that the scales were clinician-administered, they were used in their original formats and language, while the interview itself was conducted in Croatian. To avoid possible participants' exaggeration of symptoms with an aim to obtain compensation, we used only clinician-administered scales (28,29). PTSD symptomatology was assessed by psychiatrists licensed for CAPS administration (18). Since anxious and depressive symptoms are part of the PTSD clinical presentation, HAM-A and the HAM-D-17 were also applied by examiners licensed for HAM-A and HAMD-17 scales.

The CAPS is a structured interview that categorically diagnoses and measures the severity of PTSD symptoms as defined by the DSM-IV-TR. To meet the criterion of PTSD, a particular symptom has to have the sum of the frequency (score 0="none of the time" to 4="most of the time") and intensity scores (scale 0="none" to 4="extreme") (19). Inter-rater reliability for CAPS is high, ranging from 0.92 to 1.00 for "frequency" ratings, and 0.93 to 0.98 for "intensity" ratings; the global severity correlation is 0.89 (30). Test-retest reliability ranges from 0.90 to 0.98 for 17-item core symptom scale (18). Strong convergent validity has been demonstrated against the Structured Clinical Interview for DSM-IV (SCID) PTSD module (0.83) (31).

HAM-A and HAM-D-17 are the most widely used clinician-administered anxiety and depression assessment scales. The used HAM-A has 14 items and the severity of anxiety is graded as follows: normal (0-13 points), mild (14-17 points), moderate (18-24 points), and severe (≥25 points). The used HAM-D-17 has 17 items and the severity of depression is graded as follows: normal (0-6 points), mild (7-16 points), moderate (17-24 points), and severe (≥25 points). Scores of ≥17 on HAM-A and HAM-D-17 scales are usually used as a marker of clinically relevant levels of anxious/depressive symptoms (25,26). Although HAM-D-17 is not validated in Croatia, it is translated to Croatian by one of the authors (NM) (32). All examiners in our study are licensed and highly experienced in applying the English versions of all scales, and this mode of evaluation is in concurrence with psychiatric scientific practice in our country.

For general demographic data, as well as nutritional and life-style habits, we used a validated structured interview (27). During the interview, an experienced nutritionist/biochemist explained the criteria for a good quality diet to all participants according to recommendations (27). Variables specific for the study (sleep duration, loss of a close person) were included in the questionnaire.

Blood sample collection

Vein blood samples were taken from the left forearm between 8 and 9 AM after an overnight fast, and collected into 5 mL vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). They were centrifuged at 3000 rpm for 10 minutes at 15°C; the serum was separated and stored at -20°C until further analysis.

Fatty acids analysis

The fatty acids analysis was undertaken in one batch after all blood samples had been collected. Total lipids were extracted from serum with a mixture of isopropanol/chlorophorm (1.5:1 v/v) (33). Trans-esterification of fatty acids to methyl esters was performed according to International Standard ISO 5509:2000(E) (34), and the composition of fatty acid methyl esters was analyzed by a validated method ISO 5508:1990(E) (35). Gas chromatography was performed on SRI 8610C Gas chromatograph (SRI Instruments Chromatography Systems, Torrance, CA, USA), equipped with a flame ionization detector and a capillary column 007-CW (Quadrex Corporation, Woodbridge, CT, USA), length 60 m, i.d. 0.25 mm, and film thickness 0.25 μm. Hydrogen was used as a carrier gas at a flow of 60 mL/min in a split mode. The initial oven temperature of 150°C was maintained for 3 minutes then increased to 220°C at a rate of 8°C/min, and the final temperature was kept constant for 30 minutes. The injector and detector temperatures

were 250°C and 260°C, respectively. Fatty acid methyl esters were identified by comparison of their retention times to those of known reference standards (Supelco Inc, Bellefonte, PA, USA). Reproducibility and equimolar response experiments were performed with methyl heptadecanoat as internal and/or external standard, and the full procedure was applied to duplicate samples. Data were collected and analyzed using Peak Simple 3D, Version 2.97 (SRI Inc, Torrance, CA, USA). The results are reported as weight percent (wt %) of total fatty acids, and certain ratios of interest were calculated.

Statistical analysis

Data were analyzed with statistical package SPSS 19.0 (IBM Corp, Armonk, NY, USA), apart from structural equation modeling, which was undertaken using the SPSS Amos 22.0 (IBM Corp). Depending on the data distribution, quantitative data were described by mean and standard deviation (SD) or median and interquartile range. For categorical data, absolute numbers and percentages were used. Pearson correlation coefficient was used to estimate correlations between psychological scales and between fatty acids and psychological scales after eliminating the outliers and ln-transforming the variables whose distribution significantly deviated from the normal. Outliers ($n_{outliers}$ from 0 to 2) were defined as extreme scores (mean ±3SD), which distorted the size of the correlation coefficient. In parallel with Pearson, Kendall tau correlation was used as sensitivity analysis of data manipulation. Demographic/lifestyle variables possibly causing spurious correlations of PUFA with PTSD severity were identified by literature search (ie, age, BMI), or by Kendall correlation analysis (ie, preferred oil, fish, dairy product consumption, and sleeping). Additionally, smoking status, physical activity, and overall diet quality were also included in the set of possible confounders. Independent associations of fatty acids with scores on PTSD severity scales that were confirmed as significant during partial correlation analysis were further investigated in a series of multivariate general linear models (GLM) using scores on CAPS, HAM-A, or HAM-D-17 scales as dependent variables. In all cases, the assumptions for applying multivariate GLM were checked by testing the normality of dependent variables distribution (assumption of multivariate normality) and Box'M statistics (assumption of homogeneity of variance-covariances matrices).

Since we could include up to three independent variables in a multivariate model (due to a sample size limitations), modeling was performed in several steps. First, independent association between a fatty acid (EPA, AA/EPA, EPA/DHA) level and PTSD severity was evaluated using two additional covariates: a) a variable for which it was reasonable to assume that it influenced the profile of fatty acids: preferred oil, fish, and dairy products consumption or sleep duration; and b) n-6 PUFA levels that compete for the same metabolic pathways with n-3 PUFA (in models with EPA and EPA/DHA). The main effect of n-6 PUFA was also included as a covariate in the model with AA/EPA, since EPA level was the primary determinant of the magnitude of AA/EPA in our sample, due to a marked n-3 PUFA deficiency (36). Covariates that demonstrated significant multivariate effects: EPA, AA/EPA, EPA/DHA, and dairy products consumption; as well as BMI and age, which were selected based on previous studies (37), were introduced in the second analysis step in which fatty acid levels, dairy products consumption, and BMI were chosen as independent variables with the strongest multivariate effects. Finally, three multivariate models of PTSD severity were formed, using BMI, dairy products consumption, and either EPA, AA/EPA, or EPA/DHA as covariates. The Bootstrap method was used to estimate the robustness of regression coefficients.

In addition to multivariate analysis, we used structural equation modeling (SEM) (38). In this model, we defined PTSD as the underlying phenomenon measured by three psychological scales and introduced it in the model as the latent (unobserved) variable. AA/EPA ratio was the strongest predictor in multivariate analysis, with EPA being the strongest determinant of the ratio in n-3 PUFA deficiency conditions. Therefore, either AA/EPA or both: AA and EPA were introduced as exogenous variable(s) in the model, together with the dairy products consumption, which was included based on multivariate analysis results and indications from literature (39,40). We modeled both the direct (exogenous variable) and the indirect (covariate of fatty acids) influence of dairy products consumption on PTSD in the model. For multiple testing we used Bonferroni's correction, which incorporates correlation structures between multiple contrasts and multiple variables (41,42). Unless otherwise stated, *P* values <0.05 were considered significant.

RESULTS

Demographic and lifestyle characteristics

The majority of participants had a secondary school education level, sedentary lifestyle, and slept less than 5 hours/night. In food preparation they most commonly used sunflower oil, consumed dairy products a few times a week,

and presumed to have generally good or moderate overall diet quality (Table 1**).**

TABLE 1. Demographic and clinical characteristics of participants (n = 62)*

Characteristic	
Age (years), mean (SD)	47.1 ± 5.9
Body mass index (kg/m^2), mean (SD)	28.9 ± 4.5
Psychological scale scores, mean (SD)	
CAPS	69.2 ± 9.8
HAM-A	16.5 ± 2.2
HAM-D-17	13.3 ± 4.0
Level of education†, n (%)	
elementary school (≤8 y)	7 (11.3)
secondary school (>8≤12 y)	42 (67.7)
high school (>12 y)	6 (9.7)
Smoking status†, n (%)	
never	16 (25.8)
former	18 (29.0)
current	21 (33.9)
Consumption of fish and seafood†, n (%)	
<1/week	40 (51.6)
1/week	12 (19.4)
>1/week	3 (4.8)
Consumption of dairy products†, n (%)	
<1/week	9 (14.5)
a few times a week	39 (62.9)
>1/d	7 (11.3)
Physical activity†, n (%)	
sedentary (≤1/mo)	26 (42)
intermediate (2-3/week)	23 (37.1)
sufficient (≥3/week)	6 (9.7)
Oil type†, n (%)	
sunflower	40 (64.5)
olive	6 (9.7)
other	9 (14.5)
Overall diet quality†, n (%)	
very good	3 (4.8)
good	28 (45.2)
moderate	22 (35.5)
low	2 (3.2)
Sleep duration†, n (%)	
<5 h/night	29 (46.7)
6-8 h/night	25 (40.3)
>8 h/night	1 (1.6)
Loss of a close person†, n (%)	
no	19 (30.6)
yes	36 (58.1)

*Abbreviations: CAPS – Clinician-Administered PTSD Scale; HAM-A – Hamilton Anxiety Rating Scale; HAM-D-17 – Hamilton Depression Rating Scale-17.
†The sum does not add up to total because of 7 missing values.

Psychometric characteristics

The patient who entered the study with the least score had 52 points on CAPS. On HAM-A scale, 20 participants had over 17 points, indicating clinically relevant level of anxiety, although according to DSM-IV-TR they did not meet the diagnostic criteria for any other anxious disorder except PTSD. Similarly, 10 participants had clinically relevant level of depressive symptoms but did not meet the DSM-IV-TR criteria for current major depressive disorder. Based on the severity of clinical presentation, these patients could be classified as moderately anxious or moderately depressed. Comparable significant correlations were identified between CAPS and HAM-A scores (r = 0.901, 95% confidence interval [CI] 0.865-0.935; $P < 0.001$), and CAPS and HAM-D-17 scores (r = 0.868, 95% CI 0.736-0.943; $P < 0.001$).

Fatty acid composition

The composition of fatty acids in serum lipids was expressed as the ratio of a certain fatty acid in total fatty acids pool (Table 2). Fatty acids were composed mainly of PUFA (almost 39%), among which linoleic acid prevails (LA, C18:2n-6). Consequently, a high n-6/n-3 ratio was calculated. Actually both, LA and AA, contributed to this ratio as revealed by high AA/EPA and AA/DHA ratios. Saturated fatty acids made up approximately 36% and monounsaturated fatty acids the rest.

TABLE 2. Fatty acid composition (wt %) of serum lipids in patients with posttraumatic stress disorder. All values are expressed as median and interquartile range

Fatty acids	Percentage
SFA	36.4 (34.4-39.0)
MUFA	22.6 (20.6-26.5)
PUFA	39.4 (35.0-44.1)
Total n-6 PUFA	38.8 (34.1-43.1)
Total n-3 PUFA	1.4 (0.7-2.3)
AA	8.8 (7.3 -11.4)
EPA	0.1 (0.1-0.4)
DHA	0.5 (0.2-1.0)
n-6/n-3 PUFA	30.0 (15.8-52.3)
AA/EPA	53.9 (29.3-129.7)
AA/DHA	17.6 (7.7-38.9)
EPA/DHA	0.5 (0.1-0.9)

*Abbreviations: PTSD – posttraumatic stress disorder; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; AA – arachidonic acid (C20:4n-6); EPA – eicosapentaenoic acid (C20:5n-3); DHA – docosahexaenoic acid (C22:6n-3).

Associations between serum fatty acid levels and psychometric characteristics

Significant negative correlations were found between plasma EPA levels and scores on CAPS, HAM-A, and HAM-D-17 scales ($\tau = -0.326$, $P < 0.001$; $\tau = -0.304$, $P = 0.001$; $\tau = -0.345$, $P < 0.001$, respectively; corrected significance level $\alpha = 0.016$) (Table 3). Also, there were significant negative correlations between plasma EPA/DHA ratio and scores on HAM-A and HAM-D-17 scales ($\tau = -0.224$, $P = 0.015$; $\tau = -0.179$, $P = 0.047$), whereas correlation between plasma EPA/DHA ratio and CAPS score was borderline. Contrary to EPA and EPA/DHA, AA/EPA ratio was significantly positively correlated with psychological scales scores ($\tau = 0.345$, $P < 0.001$; $\tau = 0.348$, $P = 0.001$; $\tau = 0.346$, $P < 0.001$, respectively).

Levels of other analyzed fatty acids, or their ratios, were not significantly correlated with posttraumatic, anxiety, or depressive symptomatology scores (P ranging from 0.208 to 0.917). Likewise, in order to assess the possibility that some of these "correlations" were distorted by the effect of the confounding variable, we performed partial correlation analysis between PTSD severity (measured as scores on CAPS, HAM-A, and HAM-D-17 scales) and fatty acids level. None of the partial correlations was in contradiction with zero correlation finding. In particular, none of the non-significant fatty acid correlations was found to be significant

TABLE 3. Correlations between fatty acid levels and posttraumatic stress disorder symptoms, n = 62

	CAPS		HAM-A		HAM-D-17	
Measure	τ	$P^{\dagger}$	τ	P	τ	P
SFA	-0.155	0.080	-0.201	0.028	-0.066	0.464
MUFA	0.082	0.355	0.069	0.450	0.059	0.510
PUFA	0.053	0.551	0.109	0.236	0.036	0.689
n-6 PUFA	0.058	0.511	0.116	0.208	0.039	0.660
n-3 PUFA	-0.075	0.394	-0.025	0.782	-0.071	0.428
AA	0.037	0.679	0.085	0.354	0.002	0.981
EPA	-0.326	<0.001	-0.304	0.001	-0.345	<0.001
DHA	-0.069	0.436	-0.010	0.917	-0.067	0.457
n-6/n-3 PUFA	0.084	0.343	0.069	0.450	0.075	0.407
AA/EPA	0.345	<0.001	0.348	<0.001	0.346	<0.001
AA/DHA	0.085	0.336	0.054	0.559	0.070	0.435
EPA/DHA	-0.169	0.056	-0.224	0.015	-0.179	0.047

*Abbreviations: PTSD – posttraumatic stress disorder; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; AA – arachidonic acid (C20:4n-6); EPA – eicosapentaenoic acid (C20:5n-3); DHA – docosahexaenoic acid (C22:6n-3); CAPS – Clinician-Administered PTSD Scale; HAM-A – Hamilton Anxiety Rating Scale; HAM-D-17 – Hamilton Depression Rating Scale-17.
†Corrected significance level $\alpha = 0.016$; τ - Kendall's tau correlation coefficient.

TABLE 4. Multivariate general linear models of a severity of PTSD symptoms (measured by scores on CAPS, HAM-A, and HAM-D-17 scales) with covariates: fatty acid level and BMI; and a fixed factor: dairy products consumption, n = 62

	Predictors strength		
Covariates	Wilks' lambda	P-value†	Partial Eta squared
EPA	0.706	0.002	0.294
Dairy products consumption	0.756	0.061	0.131
BMI	0.857	0.087	0.143
AA/EPA	0.629	<0.001	0.371
Dairy products consumption	0.726	0.033	0.148
BMI	0.876	0.131	0.124
EPA/DHA	0.833	0.051	0.167
Dairy products consumption	0.727	0.033	0.142
BMI	0.844	0.065	0.156

*Abbreviations: PTSD – posttraumatic stress disorder; BMI – body mass index (kg/m^2); AA – arachidonic acid (C20:4n-6); EPA – eicosapentaenoic acid (C20:5n-3); DHA – docosahexaenoic acid (C22:6n-3); CAPS – Clinician-Administered PTSD Scale; HAM-A – Hamilton Anxiety Rating Scale; HAM-D-17 – Hamilton Depression Rating Scale-17.
†Significance level 0.05.

even after we included age, BMI, smoking status, physical activity, sleeping duration, overall diet quality, and type of oil, fish, or dairy products consumption as a confounder. At the same time, all significant correlations of fatty acids remained significant (Table 3**).**

Independent correlations between EPA, AA/EPA, EPA/DHA, and PTSD severity were further assessed through several steps by multivariate GLMs, with scores on CAPS, HAM-A, and HAM-D-17 scales entered as dependent variables.

Models that included covariates: fatty acids (EPA, AA/EPA or EPA/DHA, and n-6 PUFA levels which compete with n-3 PUFA levels) and a fixed factor (a potential confounder related to dietary/lifestyle quality such as oil preference, consumption of dairy products, fish consumption, or sleep duration) confirmed that PTSD severity was affected by EPA (Wilks'Λ = 0.763-0.805, $P = 0.006$ to 0.018, η_p 0.195-0.237), AA/EPA (Wilks'Λ = 0.699-0.757, $P = 0.004$, η_p 0.243-0.301), and dairy products consumption (Wilks'Λ = 0.760-0.791, $P = 0.045$-0.088, η_p 0.128-0.111). On the contrary, no other dietary/lifestyle variable was significant ($P = 0.362$-0.633). EPA/DHA ratio also did not demonstrate significant multivariate effect (Wilks'Λ = 0.901-0.960, $P = 0.185$-0.596, η_p 0.040-0.099) but was nevertheless introduced to further analysis.

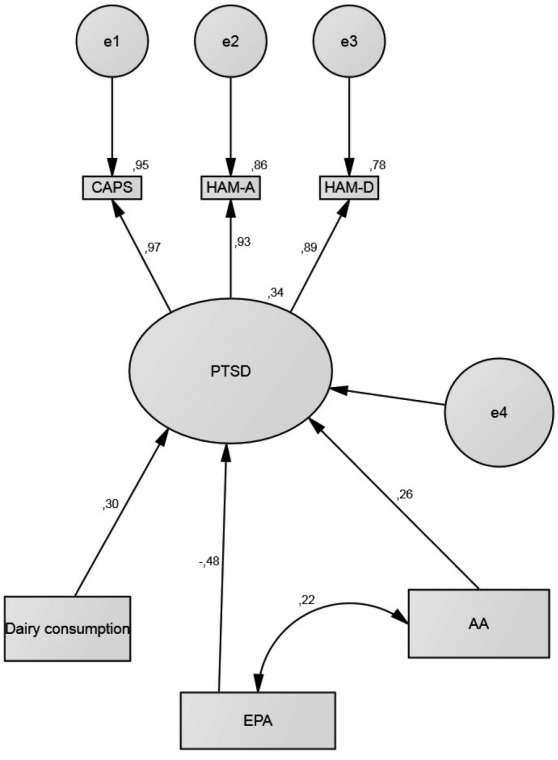

FIGURE 1. Best-fitting structural equation model of post-traumatic stress disorder (PTSD) severity showing relationships between unobserved endogenous (PTSD) and observed endogenous (Clinician-Administered PTSD Scale, Hamilton Anxiety Rating Scale, and Hamilton Depression Rating Scale-17) and exogenous (dairy products consumption, eicosapentaenoic acid, and arachidonic acid levels) variables. The figure also shows unobserved exogenous error (e1-e4) variables, percentage of variance explained by dependent variables (rectangles and ellipse), and correlation between variables (arrows).

The next stage of analysis consisted of running three separate multivariate GLMs. The strongest effect was observed for AA/EPA, followed by EPA and EPA/DHA. The multivariate effect of dairy products consumption was also significant, whereas the effect of BMI exhibited borderline significance (Table 4).

Finally, a structural equation model (SEM) was built in order to construct a causal model of the relationships among variables. The model was built on complete data of 62 participants and had the best fit (Figure 1). The departure of the data from the model was not significant ($\chi^2_8 = 6751$, $P = 0.564$), thereby indicating a good model fit, as confirmed by Root Mean Square Error of Approximation (RMSEA) fit index <0.001 (90% CI from 0 to 0.138). All of the paths made significant contributions to the success of the model, $P < 0.05$ (unlike covariances between dairy products consumption and PUFA). The percentage of variance explained by PTSD indicated that CAPS was the most precise measurement scale (97%), followed by HAM-A (86%) and HAM-D-17 (78%). Exogenous variables AA, EPA, and dairy products consumption in total explained 34% of PTSD variance. Correlation coefficients indicated plausible biological interactions. EPA moderately and negatively correlated with PTSD severity, whereas AA and dairy products consumption were weakly and positively correlated. DHA, on the other hand, was not identified as a significant covariant.

DISCUSSION

Fatty acids and the severity of PTSD symptoms

Our study revealed an inverse correlation between EPA, AA/EPA, and EPA/DHA levels and the severity of the PTSD symptoms. No significant correlations were found between AA and DHA levels, or any other analyzed fatty acid, and the score on the psychiatric scales. Therefore, the AA/EPA and EPA/DHA ratios could have corre-

lated with PTSD symptoms solely due to EPA level, which seems to be the only fatty acid that had a significant correlation with PTSD symptoms. Our results are consistent with the report that plasma EPA level was inversely associated with the severity of depressive symptoms in elderly subjects receiving antidepressant treatment (23). Similarly, another study reported a negative correlation between EPA level and severity of depression (43). The Health 2000 Survey, which included 2608 male participants, has also shown a significant relationship between serum EPA levels and psychological distress (44).

Data on the correlation between DHA and psychiatric disorder are inconsistent. Both a significant negative correlation between DHA concentrations and the severity of social anxiety disorder (45) as well as a positive association between serum DHA level and distress scores (24) were reported.

Our results can be ascribed to low n-3 PUFA intake and/or to an altered fatty acids metabolism in patients with PTSD. Stress alters gastrointestinal motility, and in this way possibly influences fatty acids' absorption (46). On the other hand, neurotransmitter dysfunction related to a disorder process could alter the metabolism of n-3 PUFA by influencing the activities of the rate-limiting enzymes (45). The opposite mechanism is also possible. Both n-6 and n-3 PUFA compete for the same set of enzymes involved in desaturation and elongation. If n-6 PUFA level outmatches fatty acids from n-3 series, the resulting misbalance in neuronal membranes will result in an impaired signal transduction.

Effect of dairy products consumption and BMI on the severity of PTSD symptoms

We found both a significant multivariate effect of dairy products consumption and a borderline significant multivariate effect of BMI on the severity of PTSD symptoms. Although the evidence is scarce, two studies have associated dairy products consumption with increased anxiety, stress, depression scores, and risk of cognitive decline (39,40). An extensive variety of biological, cognitive, behavioral, and social factors may underlie the relationship between BMI and depression symptoms (47,48). It was presumed that depression can lead to obesity via physiological and psychosocial pathways, including elevated stress reaction, immunological dysfunction, and hypothalamic-pituitary-adrenal (HPA) axis (47). In addition, dysfunctions in HPA axis and the immune system were also established in PTSD patients (49,50).

PTSD treatment and n-3 PUFA supplementation

Pharmaceutical treatment of PTSD dominant core symptoms and the related anxiety and depressive symptoms has been shown as successful (51-54). However, combat-related PTSD causes a stronger functional impairment and is less responsive to treatment than PTSD related to other traumas (28,29,53,54). It is worth mentioning that animal studies have confirmed that n-3 unsaturated fatty acids enhance hippocampal neurogenesis (55). In PTSD, the hippocampal volume is reduced and a long-term over-activity of HPA axis causes adjacent adaptive changes of glucocorticoid receptors with subsequently deleterious effects for the hippocampal neurons (56,57). Assuming that the animal model could be representative for humans, PTSD patients may benefit from n-3 PUFA supplementation, as it could enhance the hippocampal neurogenesis. Previous studies showed a significant increase in serum lipids concentration and associated risk factors for atherosclerosis and coronary artery disease in a sample of veterans suffering from chronic PTSD (58,59). Associations of n-3 PUFA deficit with many psychiatric disorders (1), as well as favorable effects of n-3 PUFA on the cardiovascular system, are well established (60). This makes n-3 PUFA supplementation a new potential treatment approach for psychiatric disorders, with added beneficial effect on the comorbid cardiovascular disorders.

As shown in a recent review, a number of clinical trials investigated the effects of n-3 PUFA supplementation in depressive disorder, schizophrenia, bipolar disorder, and social anxiety disorder (1). However, to date no study has investigated the supplementary effects of n-3 PUFA status in chronic, combat-related PTSD.

Limitations and strengths

Although our sample size is relatively small, the highly significant results observed by a series of different statistical approaches obviously indicate certain effects of fatty acids composition on the severity of PTSD symptoms. Further studies should gather more detailed dietary data as it is well known that dietary choices affect plasma fatty acid composition. Also, it would be of interest to make a comparison with a control group unaffected by PTSD. The cross-sectional design of our study did not allow us to determine whether the lower plasma EPA levels contributed to PTSD development or vice versa. It could also be considered a limitation that patients' last meal before blood collection was not of the same quantity and components

and it was not taken at the same time, but the patients were allowed to follow their own nutritional habits. There is no doubt that nutrition affects blood fatty acids composition both in short- and long-term. However, blood samples were taken at least 12 hours after the last meal, which means that fatty acids taken from the gut and released into the bloodstream via chylomicrons had allready been cleared out and could not have significantly affected the average fatty acid composition.

However, our study has several strong points. We studied a homogenous group of participants with clinically established PTSD who had been exposed to the same type of trauma experience. We recruited only male patients in order to exclude the influence of gender and the associated hormones on the metabolism of lipids and fatty acids. As the activity of enzymes responsible for the synthesis of EPA and DHA decreases with age, the long-chain PUFA status, notably DHA, becomes more dependent on dietary supply (23,61). Having this in mind we recruited participants younger than 65 years, thus contributing to the better homogeneity of the study sample.

In conclusion, our study showed a significant negative association between EPA levels and chronic, combat-related PTSD symptoms, thus confirming our hypothesis. Further longitudinal studies on a larger sample are necessary to affirm our results and to reveal the mechanisms underlying the relationship between plasma PUFA levels and PTSD. We suggest further investigations of n-3 PUFA supplementation in combat-related PTSD veterans since data indicate potential multiple benefits of this approach in reduction of negative consequences of the illness.

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Declaration of authorship DK contributed to data collection and analysis, made data interpretation, searched the literature, and wrote the manuscript. LBŠ collected samples and performed the analyses. AJ made statistical analysis, contributed to data interpretation and took part in writing of the manuscript. NM supervised the project, contributed to data collection, and made the critical revision of the manuscript for important intellectual content. GD contributed to study design, and made the critical revision of the manuscript for important intellectual content. ID contributed to study design, performed fatty acid analysis, took part in writing of the manuscript, and coordinated the project. All authors read and approved the final version of the manuscript.

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