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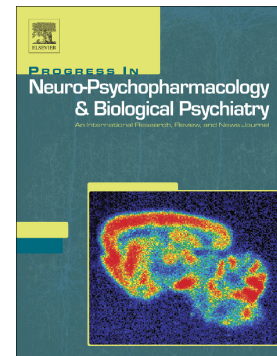
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## Accepted Manuscript

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**Eicosapentaenoic and docosahexaenoic acids have different effects on peripheral phospholipase A2 gene expressions in acute depressed patients**

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**Running title:** EPA vs. DHA in PLA2 gene expression

**Author Contributions:** Prof. Su created the concept, designed the study, received the research funding and prepared and revised the manuscript. Drs. Chang & Guu performed the clinical assessments and prepared the manuscript. Profs. Su, Walczewska, Gałeczki and Pariante designed the study, performed the literature search and manuscript preparation and revision. All the authors have full access to all the data and take responsibility for the integrity and accuracy of this study.

**Keywords:** Omega-3 polyunsaturated fatty acids (n-3 PUFAs), Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), Major depressive disorder (MDD), Phospholipase A2 (PLA2), Gene expressions

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**Abstract**

**Introduction:** Omega-3 polyunsaturated fatty acids (PUFAs) have been proven critical in the development and management of major depressive disorder (MDD) by a number of epidemiological, clinical and preclinical studies, but the molecular mechanisms underlying this therapeutic action are yet to be understood. Although eicosapentaenoic acid (EPA) seems to be the active component of omega-3 PUFAs' antidepressant effects, the biological research about the difference of specific genetic regulations between EPA and docosahexaenoic acid (DHA), the two main components of omega-3 PUFAs, is still lacking in human subjects.

**Methods:** We conducted a 12-week randomized-controlled trial comparing the effects of EPA and DHA on gene expressions of phospholipase A2 (cPLA2) and cyclo-oxygenase-2 (COX2), serotonin transporter (5HTT), and Tryptophan hydroxylase 2 (TPH-2) in 27 MDD patients. In addition, the erythrocyte PUFA compositions and the candidate gene expressions were also compared between these 27 MDD patients and 22 healthy controls.

**Results:** EPA was associated with a significant decrease in HAM-D scores (CI: -13 to -21,  $p < 0.001$ ) and significant increases in erythrocyte levels of EPA (CI: +1.0% to +2.9%,  $p = 0.001$ ) and DHA (CI: +2.9% to +5.6%,  $p = 0.007$ ). DHA treatment was associated with a significant decrease in HAM-D scores (CI: -6 to -14,  $p < 0.001$ ) and a significant increase in DHA levels (CI: +0.2% to +2.3%,  $p = 0.047$ ), but not of EPA levels. The cPLA2 gene expression levels were significantly increased in patients received EPA (1.9 folds,  $p = 0.038$ ), but not DHA (1.08 folds,  $p = 0.92$ ). There was a tendency for both EPA and DHA groups to decrease COX-2 gene expressions. The gene expressions of COX-2, cPLA2, TPH-2 and 5-HTT did not differ between MDD cases and healthy controls.

**Conclusions:** EPA differentiates from DHA in clinical antidepressant efficacy and in upregulating cPLA2 gene regulations, which supports the clinical observation showing the superiority of EPA's antidepressant effects.

**Trial Registration:** ClinicalTrials.gov identifier: NCT02615405

## Introduction

Omega-3 polyunsaturated fatty acids ( $\omega$ -3 or n-3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential nutritional compounds with potential preventive and therapeutic effects against depression (Lin *et al.*, 2012; Sarris *et al.*, 2015; Song *et al.*, 2016; Su, 2015a; Su, 2015b; Su and Balanzá-Martínez, 2013; Su *et al.*, 2014; Su *et al.*, 2015; Su *et al.*, 2013). Patients with major depressive disorder (MDD) have lower levels of omega-3 PUFAs (Lin *et al.*, 2010), and societies that consume a larger amount of omega-3 PUFAs have a lower prevalence of MDD (Hibbeln, 1998; Tanskanen *et al.*, 2001). More importantly, many clinical trials and meta-analyses (Lin *et al.*, 2012; Lin and Su, 2007; Martins *et al.*, 2012; Sublette *et al.*, 2011), if not all (Bloch and Hannestad, 2012; Marangell *et al.*, 2003; Silvers *et al.*, 2005), have shown that omega-3 PUFAs have antidepressant effects.

The hypothesized mechanisms underlying PUFAs' antidepressant effects are their action on neurotransmission and neuroinflammation (Song *et al.*, 2016; Su, 2009, 2012, 2015b). DHA has been shown to regulate neurotransmitters function, including serotonin, norepinephrine and dopamine (Chalon, 2006; Kudas *et al.*, 2004; Zimmer *et al.*, 2002), which is of relevance to the traditional monoamine theory of depression. On the other hand, EPA is important in regulating immune function by antagonizing membrane arachidonic acid (AA, an n-6 PUFA), reducing prostaglandin E2 (PGE2) synthesis (Farooqui *et al.*, 2006), and preventing the response to inflammatory stimuli (Lu *et al.*, 2010; Moon *et al.*, 2007; Moon and Pestka, 2003), which is link to the promising inflammation theory of depression. Cytosolic phospholipase A2 (cPLA2) and cyclo-oxygenase-2 (COX2) are the two key enzymes of the PUFA metabolism and PGE2 synthesis and have been proposed to be critical in modes of action for mood stabilizers

in animal studies (Bosetti *et al.*, 2002; Rao *et al.*, 2007; Rao *et al.*, 2008). The mRNA expressions of the genes encoding for COX-2 were significantly increased in the peripheral blood cells of depressed patients (Galecki *et al.*, 2012). In addition, a genetic variation, the BanI GG polymorphism, on cPLA2 has been reported to be associated with major depressive disorder (Pae *et al.*, 2004), which has been replicated in a another population of depression induced by interferon-alpha therapy (Su *et al.*, 2010).

Despite the fact that DHA is the major omega-3 PUFA in the brain, EPA seems to be the most active component of omega-3 PUFAs' antidepressant effects (Lin *et al.*, 2012; Lin and Su, 2007; Martins *et al.*, 2012; Su *et al.*, 2013; Sublette *et al.*, 2011). Our recent study further supports this notion by showing that EPA, but not DHA, pre-treatment significantly decreased the incidence of interferon- $\alpha$ -induced depression in HCV patients (Su *et al.*, 2014). Indeed, clinical trials using only DHA monotherapy as antidepressant strategy have shown conflicting findings: Marangell *et al.* (Marangell *et al.*, 2003) found no benefit over placebo for 2 g/day DHA, but Mischoulon *et al.* (Mischoulon *et al.*, 2008) found a dose-response effect supporting 1 g/day as superior to 2 g/day or 4 g/day, though the latter study was limited by the lack of a placebo arm. However, the mechanistic understanding about the difference in clinical effectiveness between EPA and DHA in human subjects is still lacking.

To our knowledge, there are no studies examining the changes of gene expression on serotonin transporter (5HTT), Tryptophan hydroxylase 2 (TPH-2), cPLA2 and COX2 in patients receiving omega-3 PUFAs as antidepressant monotherapy. Therefore, we have specifically conducted a 12-week EPA and DHA interventional study to investigate their clinical and biological effects in patients with acute episode of MDD. Our hypothesis is that EPA and DHA might have different effects on these gene expressions.

## Method

### *Subjects*

All the subjects were referred from the outpatient psychiatric department at the China Medical University Hospital, Taichung, Taiwan, where the Institutional Review Board approved the study. Eligible participants were those who met the following criteria: (1) the diagnostic criteria of DSM-IV for major depressive disorder, (2) 18 to 65 years old, (3) pre-study ratings of 18 or greater on the 21-item Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960) and “mildly” to “moderately” ill on the Global Severity Item of the Clinical Global Impression (GCI) scale, (4) physically healthy on medical history and physical examination and laboratory parameters within normal limits, (5) haven’t received any psychiatric treatment in 2 weeks, (6) competent to receive a full explanation of the study and give written informed consent. The exclusion criteria for patients were: (1) a recent or past history of other axis-I diagnoses besides unipolar major depression, including psychotic disorders, organic mental disorders, impulse control disorders, substance use disorder or substance abuse (last 6 months prior to the studies), and bipolar disorders; (2) axis-II diagnoses, i.e. borderline and antisocial personality disorder; (3) a notable medical comorbidity; (4) acutely suicidal ideation and attempt were noted that close monitoring such as hospitalization is necessary; and (5) regular consumption of omega-3 PUFA supplements or a habit of eating fish equal or more than 4 times per week. This study was registered at ClinicalTrials.gov under the identifier NCT02615405.

### *Study Design and Recruitment*

Before entering the study, every eligible patient will be assessed by trained psychiatrists for any psychiatric disorders as determined by the Structured Mini-International Neuropsychiatric Interview (MINI) (Sheehan *et al.*, 1998). The information of translation, validation and instruction of Taiwanese version of MINI can be accessed at the website of Taiwanese Society of Psychiatry ([www.sop.org.tw/dow\\_a.htm](http://www.sop.org.tw/dow_a.htm)). As secondary analyses of original clinical trials, we enrolled and randomized the first 30 from 40 eligible outpatients consented to participate in this biological research from a 12-week, double-blind, randomized-controlled trial, comparing the antidepressant effects of EPA (3.5 g/d) and DHA (1.75 g/d). Twenty-seven of them completed the 12-week trial and were included in the analysis. For allocation of the participants following simple double-blind randomization procedures, a computer-generated list of random numbers was used. The identical capsules were pre-packed in bottles and consecutively numbered according to the randomisation schedule by an independent nutritionist. One subject discontinued from the EPA group and 2 from the DHA group. Sociodemographic factors, including gender, age, education, marital status, as well as the past psychiatric history, substances use history and family history will be recorded. All patients who agreed to participate in this study provided their signed written informed consent before enrolment.

MDD patients were assigned randomly to take of 5 identical capsules of EPA (3.5 g/d) or DHA (1.75 g/d) in a double-blind fashion for 12 weeks. The experimental capsules containing concentrated EPA (solely 700-mg EPA) or DHA (solely 350-mg DHA); they weighted 1000 mg, were deodorized with orange flavour, and supplemented with tertiary-butyl hydroquinone (0.2 mg/g) and tocopherols (2 mg/g) as antioxidants.

The sources of EPA and DHA were from anchovy fish body oil (purchased from AK BioTech, Ulsan, Korea) and algal vegetable (purchased from DSM Nutritional Products, Basel, Switzerland), respectively. The treatment protocol is designed to investigate potential different effects of EPA and DHA in therapeutic and biological effects in patients with major depression.

The judgment of concomitant medications for anxiety and insomnia or discontinuation from this trial for re-allocation to antidepressant drugs will be based on physicians' clinical judgment. Depressive symptoms (the 21-item HAM-D and the 21-item Beck Depression Inventory, BDI) (Beck *et al.*, 1996; Hamilton, 1960) were assessed for clinical outcomes before therapy (week 0), and at week 1, 2, 4, 8, and 12. The dietary habits will be assessed with the Taiwanese version of Mini Nutrition Assessment (MNA) (Tsai *et al.*, 2007).

To compared the baseline gene expression profiles between cases and controls, twenty-two healthy controls from eligible volunteers were enrolled after exclusion of any psychiatric disorders as determined by the Structured Mini-International Neuropsychiatric Interview (MINI) (Sheehan *et al.*, 1998). Sociodemographic factors, including gender, age, education, marital status, as well as the past psychiatric history, substances use history, and family history were recorded.

#### *Laboratory Methods*

Blood samples were obtained from venous blood between 08:30 and 09:30 after an overnight fasting at weeks 0 and 12. The blood tissues were prepared in 24 hours, separating erythrocyte for analysis for individual fatty acids by gas chromatography and

RNA extracted from peripheral blood mononuclear cells (PBMCs) for gene expression. Laboratory measures were conducted on coded samples by research workers who were blind to subjects' information.

Fatty acid composition of erythrocyte membranes was analyzed and the level of individual fatty acid was measured with gas chromatography of methyl esters (Lipid Standards, FAME, Sigma Co., St. Louis, MO, USA). The detailed step-by-step procedures have been published and described elsewhere (Chang *et al.*, In Press; Chiu *et al.*, 2003; Jadoon *et al.*, 2012; Su *et al.*, 2003; Su *et al.*, 2008; Su *et al.*, 2010; Su *et al.*, 2014). Fatty acid profiles were identified by comparing the retention times with those of appropriate standard fatty acid methyl esters. The levels of each fatty acid were expressed as a percentage of total fatty acids. Researchers who participated in the laboratory were blind to the information of coded samples.

Gene expression of cPLA2 (PLA2G4A), COX-2, and 5-HTT was determined by qRT-PCR analysis with the ABI PRISM® Step One Plus™ (Applied Biosystems, Foster City, CA). Total RNA from PBMCs was isolated using the RNeasy Mini Kit (Qiagen, CA, USA) according to the manufacturer's method. RNA concentration was determined by NanoDrop Spectrophotometer (Nanodrop Technologies). Briefly, 100ng of total RNA was reverse transcribed with QuantiTect Reverse Transcription Kit (Qiagen Cat. No.205311), and the amplification reactions was performed in triplicate in 25 µl final volumes via SYBR Green chemistry on an ABI PRISM® Step One Plus™ (Applied Biosystems, Foster City, CA). Gene-specific primers for human were from the Qiagen Quanti Tect Primer Assay. The gene sequence (QT) and accession numbers (NM), respectively, were QT00085813 and NM\_024420 for cPLA2, QT00040586 and NM\_000963 for COX-2, QT00053886 and NM\_173353 for the TPH-2, and QT00058380 and NM\_001045 for

5-HTT. All genes were amplified according to the Real-Time Cycler conditions suggested by Qiagen. The GAPDH gene amplification was used as a reference standard to normalize the target signal. The QuantiFast SYBR Green conditions were followed by the manufacture protocol. Cycle threshold was automatically calculated by manufacturer's software, with an independent average threshold determined for each target and each plate. Individual reaction kinetics were analyzed using a five-fold dilution series of total RNA to ensure each qRT-PCR did not differ significantly from 100%. The relative quantity of mRNA expressions were estimated using  $2^{-\Delta CT}$ , where  $\Delta CT$  is defined as [ $C_T$  gene of interest -  $C_T$  gene of GAPDH] (Schmittgen and Livak, 2008; Winer *et al.*, 1999).

#### *Data Analyses and Statistics*

The SPSS statistical software (SPSS Inc., Chicago, Illinois, USA), version 15, was used to compare continuous variables between case and controls using independent-samples *t* test and to compare continuous variables before and after omega-3 PUFAs intervention using paired *t* test. The categorical clinical variables were analysed using  $\chi^2$  (chi-squared) test. *P* values less than 0.05 were considered statistically significant. Whenever appropriate, data were reported as Mean $\pm$ SD and the error bars were represented as standard error of the means (SEM) in the result figures. The ratio of the mean changess of gene expression after interventions was estimated as 30%. The power calculation was accessed by online software on powerandsamplesize.com website. The result of sample size estimation for within subjects (before and after EPA/DHA interventions) was a total sample size of 42 (21 in each group) with power of 0.9 ( $\alpha=0.05$ ).

## RESULTS

### *MDD versus healthy controls*

The participating subjects included 27 (5 male and 22 female) depressive patients with a mean age of  $45 \pm 13$  (S.D.) years, and 22 (5 male and 17 female) healthy controls with mean age of  $45 \pm 12$  (S.D.) years. **Table 1** presents sociodemographic data, the severity of depression, and the levels of EPA and DHA before and after omega-3 PUFA levels. There were no significant differences between MDD cases and healthy controls in age, gender distribution, education, and body mass index (BMI). Patients with MDD had a significantly high BDI scores. In depressive patients, the age of onset was  $35 \pm 10$  (S.D.) years and the duration of index episode was  $17 \pm 20$  (S.D.) weeks. Numbers of depressive episodes were  $1.5 \pm 0.6$  (S.D.). As compared to controls, patients with MDD had significantly lower levels of EPA (CI: -0.10 to -1.17%,  $p=0.046$ ) and DHA (CI: -1.08 to -2.81%,  $p<0.001$ ) on erythrocyte membranes before omega-3 PUFAs supplements. **Figure 1** shows the comparisons of gene expressions of cPLA2 (1a), COX-2 (1b), TPH-2 (1c) and 5-HTT (1d) in peripheral blood mononuclear cells (PBMCs). There were no significant differences between MDD patients and healthy controls in all four selected gene expressions with RNA extracted from peripheral blood mononuclear cells.

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### *EPA versus DHA treatments*

Twenty-seven patients with MDD were assigned randomly to receive 12-week intervention with EPA (n=14) or DHA (n=13) in a double-blind fashion. **Table 2** presents sociodemographic data, the severity of depression, and the levels of EPA and DHA before and after omega-3 PUFA levels. At baseline, there were no significant differences between patients and controls in age, gender distribution, marital status, education, BMI, duration of index episode, numbers of depressive episodes, HAM-D scores, and EPA and DHA levels. After 12-week omega-3 PUFAs treatments, EPA treatment was associated with significant decreased HAM-D scores (CI: -13 to -21 points,  $p < 0.001$ ) and significant increases in levels of erythrocyte EPA (CI: +1.0% to +2.9%,  $p = 0.001$ ) and DHA (CI: +2.9% to +5.6%,  $p = 0.007$ ). DHA treatment was also associated with significant decreased HAM-D scores (CI: -6 to -14 points,  $p < 0.001$ ) and an increase of DHA levels (CI: +0.2% to +2.3%,  $p = 0.047$ ), but not EPA levels (CI: -0.6% to +1.1%,  $p = 0.6$ ). As compared to DHA group, EPA treatment was associated with significant lower HAMD scores (CI: -2 to -13 points,  $p < 0.001$ ) and higher EPA levels (CI: +1.0% to +2.7%,  $p = 0.01$ ) on erythrocyte membranes at Week 12 (**Table 2**). EPA and DHA were found well tolerated in this population. No participant was withdrawn because of adverse events by investigators' decision.

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There were no significant differences between EPA and DHA groups in levels of relative expression in any of the 4 genes at baseline (before omega-3 PUFAs treatment).

**Figure 2** shows the fold changes of cPLA2 (2a), COX-2 (2b), TPH-2 (2c) and 5-HTT (2d) gene expressions after 12-week EPA or DHA treatments. MDD patients received EPA treatment did significantly change the gene expressions of cPLA2 (1.89 folds,  $p=0.038$ ), but not COX-2 (0.36 folds,  $p=0.09$ ), TPH-2 (1.18 folds,  $p=1.00$ ) and 5-HTT (1.46 folds,  $p=0.06$ ). DHA treatment did not significantly change of gene expression of any of the 4 genes of cPLA2 (1.08 folds,  $p=0.92$ ), COX-2 (0.36 folds,  $p=0.14$ ), TPH-2 (0.96 folds,  $p=0.62$ ) and 5-HTT (0.87 folds,  $p=0.82$ ). Since the fold changes of cPLA2 and COX2 in EPA and DHA groups were at the same direction, the combination of both groups (MDD patients received omega-3 PUFAs treatment for 12 weeks) did have significant changes in gene expressions on cPLA2 and COX-2, but not on TPH-2 and 5-HTT. Specifically, the gene expressions of cPLA2 increased and COX-2 decreased significantly after 12-week omega-3 PUFAs supplementation as shown in **Figure 1**.

Finally, we did the correlation analyses of changes between the score improvement in depression and the changes of levels in cPLA2, COX-2, TPH-2 and 5-HTT gene expressions and erythrocyte DHA and EPA levels. There were no significant results in these correlation analyses.

## DISCUSSION

The main finding of our current study is that EPA differentiates DHA in clinical antidepressant efficacy and in cPLA2 gene regulations. In addition, omega-3 PUFAs treatment significantly increased the cPLA2 and decreased COX-2 gene expressions, but not TPH-2 nor 5-HTT gene expressions. To our knowledge, this is the first study to demonstrate that omega-3 PUFAs as an antidepressant treatment significantly changed peripheral blood gene expressions in patients with MDD. Previous clinical trials and

meta-analyses have shown that the efficacy of omega-3 fatty acids as antidepressant might be dependent on the ratio of EPA and DHA, and have suggested that EPA, rather than DHA, might be the most active component of omega-3 PUFAs' antidepressant effects (Lin *et al.*, 2012; Lin and Su, 2007; Martins *et al.*, 2012; Su *et al.*, 2013). The different clinical antidepressant effects implied different biological pathways of EPA and DHA, and it is now supported by our current study that EPA, but not DHA, significantly increased cPLA2 gene expression after 12-week omega-3 PUFA supplement in the gene expression model.

Consistent to previous studies to show the association between cPLA2 and COX2 genetic variations and depression (Pae *et al.*, 2004; Su *et al.*, 2010), our findings further support the important role of cPLA2 and COX-2 genes in modulation of omega-3 PUFAs' antidepressant effects. In addition, as cPLA2 and COX2 have been proposed to be critical in mode of action of mood stabilizers in animal studies (Bosetti *et al.*, 2002; Rao *et al.*, 2007; Rao *et al.*, 2008), our findings replicate this important pathway in human subjects. Omega-3 PUFAs intervention significantly changed gene expressions of cPLA2 and COX-2, but not TPH-2 and 5-HTT, indicating the antidepressant effects from omega-3 PUFAs might be independent from the regulation of monoamine systems. On top of several animal studies to show that omega-3 PUFAs can regulate gene expressions that play important roles in synaptic plasticity and neuronal function (de Urquiza *et al.*, 2000; Kitajka *et al.*, 2004; Puskas and Kitajka, 2006), the gene expression regulation in our current study implied that PUFA metabolism and inflammation systems would also play a role in antidepressant actions.

Although MDD patients in EPA group have shown significantly lower HAMD scores at week 12, both EPA and DHA significantly decreased HAMD scores after 12-week

treatment. Without a placebo comparison, our current study could not rule out potential antidepressant effects from DHA. For example, a recent meta-analysis has suggested that both EPA and DHA contribute to antidepressant effects, even though that the effects of EPA are stronger (Sublette *et al.*, 2011). In our recent clinical trial to demonstrate preventive effects of omega-3 PUFAs in interferon- $\alpha$ -induced depression, we found that EPA reduces the incidence of depression while DHA delays the onset of depression (Su *et al.*, 2014). In our current study, EPA intervention increased both EPA and DHA levels. Theoretically, EPA can be metabolised into DHA, and EPA intervention can increase blood and brain levels of DHA (Brenna, 2002), which is particularly relevant in this context as we used to identify lower endogenous DHA as a risk factor for interferon- $\alpha$ -induced depression (Su, 2015a; Su *et al.*, 2010). Incidentally, in our previous animal study, we have demonstrated that a PUFAs dietary intervention is able to increase PUFAs levels in both erythrocytes and the brain, thus supporting the notion that PUFAs changes measurable in the periphery reflect changes in the brain (Huang *et al.*, 2008). These results therefore indicate possible synergistic effects of EPA and DHA on depressive symptomatology.

The secondary finding of this study is that the gene expressions of COX-2, cPLA2, TPH-2 and 5-HTT did not differ between MDD cases and healthy controls. This implied that the individual variations of gene expression between cases and controls (“between-subjects”) might be too large to be a diagnostic biomarker. In contrast, the comparisons of gene expression between pre-and post-treatment (“within-subjects”) are more suitable to apply peripheral mRNA levels as therapeutic biomarkers. In addition, our current study also replicated the significant lower levels of erythrocyte EPA and DHA in patients with MDD (Lin *et al.*, 2010). **Finally, we chose 3.5 grams of EPA**

and 1.7 grams of DHA per day because our previous studies conducted in Taiwan have shown the effective dose to be between 2.2 g/day and 4.4 g/day for EPA, and 1.2 g/day to 2.2 g/day for DHA (Su *et al.*, 2003; Su *et al.*, 2008; Su *et al.*, 2014). In addition, we chose EPA with a higher dosage than DHA because the bioavailability of EPA might be reduced after metabolizing into DHA and other forms of omega-3 PUFAs, while DHA might be more stable regarding its bioavailability. These doses are relatively high, which is consistent to the fact that the baseline dietary content of fish is much higher in Taiwan than in many Western countries (Hibbeln, 1998).

Some of the findings may appear difficult to reconcile, but this is part due to the inherent limitations of insufficient statistical powers in measuring gene expressions with large individual variations, rather than a true inconsistency within findings. For example, the significant effects of decreasing COX-2 gene expressions by omega-3 PUFAs (combining EPA and DHA) disappeared when the whole group was divided into EPA and DHA groups, although there is a tendency of decreasing COX-2 gene expressions by both EPA and DHA (Figure 2). In addition, this study is also limited by its relatively small sample size. Finally, one might argue the increased cPLA2 activity after EPA treatment seems to be unfavorable because cPLA2 could release AA. One explanation is that an increased peripheral cPLA2 activity might imply a compensation of decreased cPLA2 activity and AA levels in the brains. Unfortunately, we only measured these biomarkers from peripheral blood tissues, and we did not have brain levels for correlations. Future animal studies are warranted to investigate the potential complex biological interactions.

In conclusion, the findings from our current study revealed that EPA differentiates DHA in clinical antidepressant efficacy and in cPLA2 gene regulations, which supports

the clinical observation showing the superiority of EPA's antidepressant effects. Furthermore, the study further supports the important role of cPLA2 and COX-2 genes in omega-3 PUFAs' antidepressant effects and imply that the antidepressant effects from omega-3 PUFAs might be independent from the regulation of monoamine systems.

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**HIGHLIGHTS:**

- Despite that docosahexaenoic acid (DHA) is the major omega-3 polyunsaturated fatty acids (PUFAs) in the brain, eicosapentaenoic acid (EPA) seems to be the active component of omega-3 PUFAs' antidepressant effects. However, the mechanistic understanding about the difference in clinical effectiveness between EPA and DHA in human subjects is still lacking.
- Omega-3 PUFAs treatment significantly increased the cytosolic phospholipase A2 (cPLA2) and decreased cyclo-oxygenase-2 (COX2) gene expressions, but not tryptophan hydroxylase 2 (TPH-2) nor serotonin transporter (5HTT) gene expressions, indicating the antidepressant effects from omega-3 PUFAs might be

independent from the regulation of monoamine systems.

- EPA differentiates from DHA in clinical antidepressant efficacy and in upregulating cytosolic phospholipase A2 (cPLA2) gene regulations.
- Future animal studies are warranted to investigate the potential complex biological interactions between omega-3 PUFAs and cPLA2 genetic expressions in brains.

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Table 1. Demographic and clinical characteristics of patients of major depressive disorder (MDD) and healthy controls

	MDD (N=27)	Healthy controls (N=22)	<i>p</i> *
Age (years), Mean±SD	45 ± 13	45 ± 12	0.9
Sex (Male, %)	19%	23%	0.7**
Education (years), Mean±SD	13 ± 3	12 ± 4	0.8
Body mass index (kg/m <sup>2</sup> ), mean±SD	22 ± 4	24 ± 5	0.3
Marriage (Married, %)	67%	68%	0.9**
Family History of MDD (%)	26%	0%	0.010**
Baseline BDI scores, Mean±SD	26 ± 11	5 ± 4	<0.001
HAMD scores, Mean±SD			<0.001
Week 0	26 ± 5		
Week 12	12 ± 9		
EPA levels (%), Mean±SD			
Week 0	2.82 ± 1.00	3.42 ± 1.03 <sup>^</sup>	0.046 <sup>^</sup>
Week 12	3.77 ± 1.47 <sup>#</sup>		0.016 <sup>#</sup>
DHA levels (%), Mean±SD			
Week 0	3.56 ± 0.93	5.50 ± 1.81 <sup>^</sup>	<0.001 <sup>^</sup>
Week 12	5.03 ± 1.89 <sup>#</sup>		0.001 <sup>#</sup>

\* Differences were compared by the *Mann-Whitney* test unless otherwise specified. E.g. \*\* indicates the results of  $\chi^2$  (chi-squared) tests. <sup>^</sup> indicates comparisons between MDD versus controls. <sup>#</sup> indicates comparisons between before and after treatment. Difference was considered statistically significant if a *p*-value was equal to or smaller than 0.05.

Abbreviations: MDD, major depressive disorder; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HAMD, the 21-item Hamilton Depression Rating Scale; BDI, Beck Depression Inventory

Table 2. Demographic and clinical characteristics of MDD patients received EPA and DHA

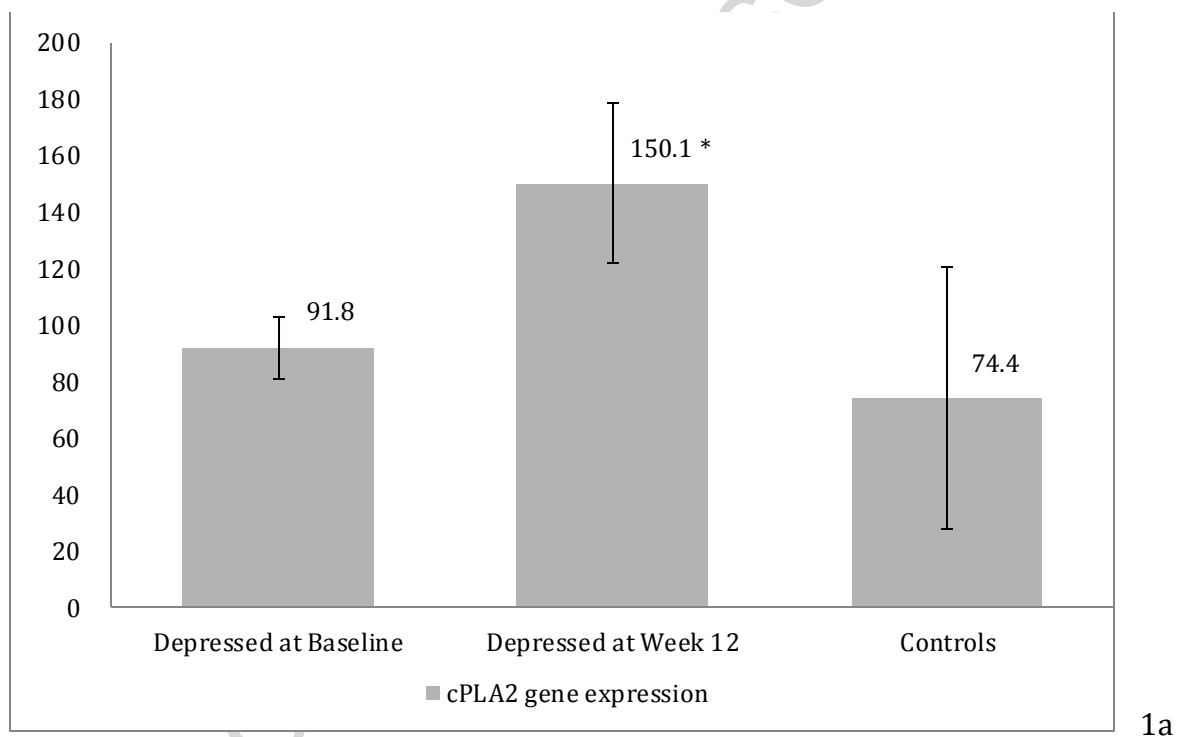
	EPA (N=14)	DHA (N=13)	<i>p</i> *
Age (years), Mean±SD	44 ± 14	46 ± 12	0.7
Sex (Male, %)	21%	15%	0.7**
Education (years), Mean±SD	13 ± 3	12 ± 3	0.3
Body mass index (kg/m <sup>2</sup> ), mean±SD	23 ± 4	21 ± 3	0.1
Marriage (Married, %)	57%	77%	0.3**
Index episode (weeks), Mean±SD	18 ± 13	16 ± 25	0.8
Numbers of episodes, Mean±SD	1.4 ± 0.5	1.6 ± 0.7	0.3
Family History of MDD (%)	36%	15%	0.2**
HAMD scores, Mean±SD			
Week 0	26 ± 5	26 ± 6	0.9
Week 12	8 ± 9	16 ± 7	0.017
EPA levels (%), Mean±SD			
Week 0	2.65 ± 0.88	3.01 ± 1.09	0.4
Week 12	4.67 ± 1.23	2.80 ± 1.03	0.001
DHA levels (%), Mean±SD			
Week 0	3.45 ± 1.00	3.67 ± 0.88	0.6
Week 12	5.22 ± 1.98	4.83 ± 1.82	0.3

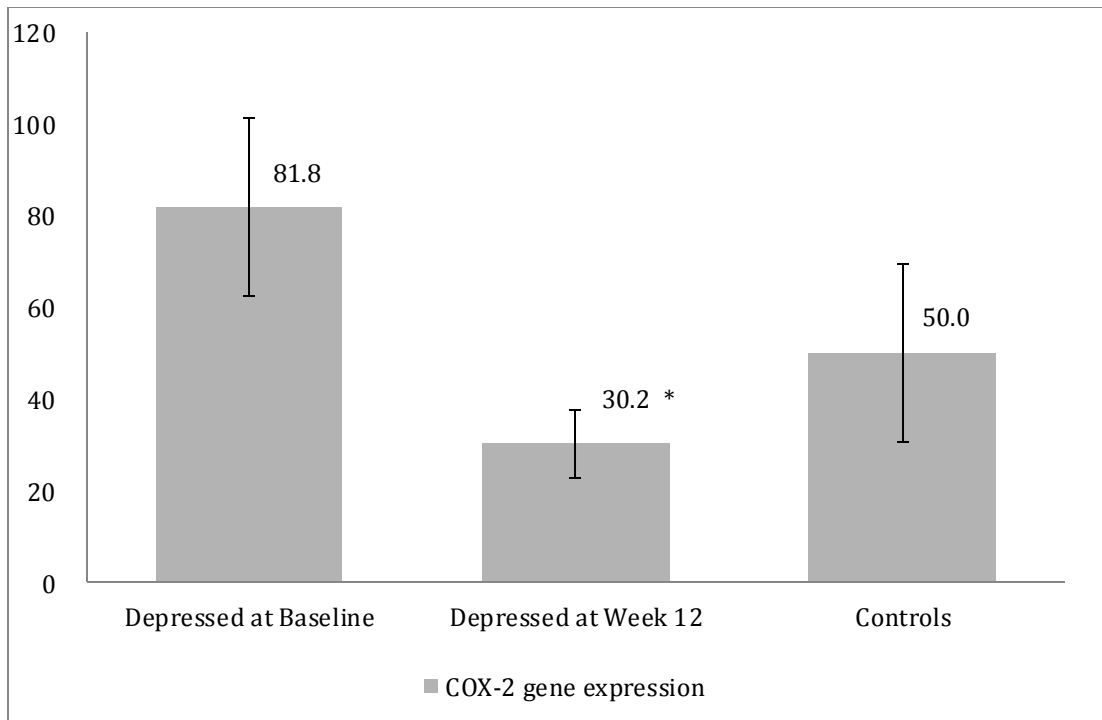
\* Differences were compared by the *Mann-Whitney* test unless otherwise specified. E.g.

\*\* indicates the results of  $\chi^2$  (chi-squared) tests. ^ indicates comparisons between MDD versus controls. # indicates comparisons between before and after treatment. Difference was considered statistically significant if a *p*-value was equal to or smaller than 0.05.

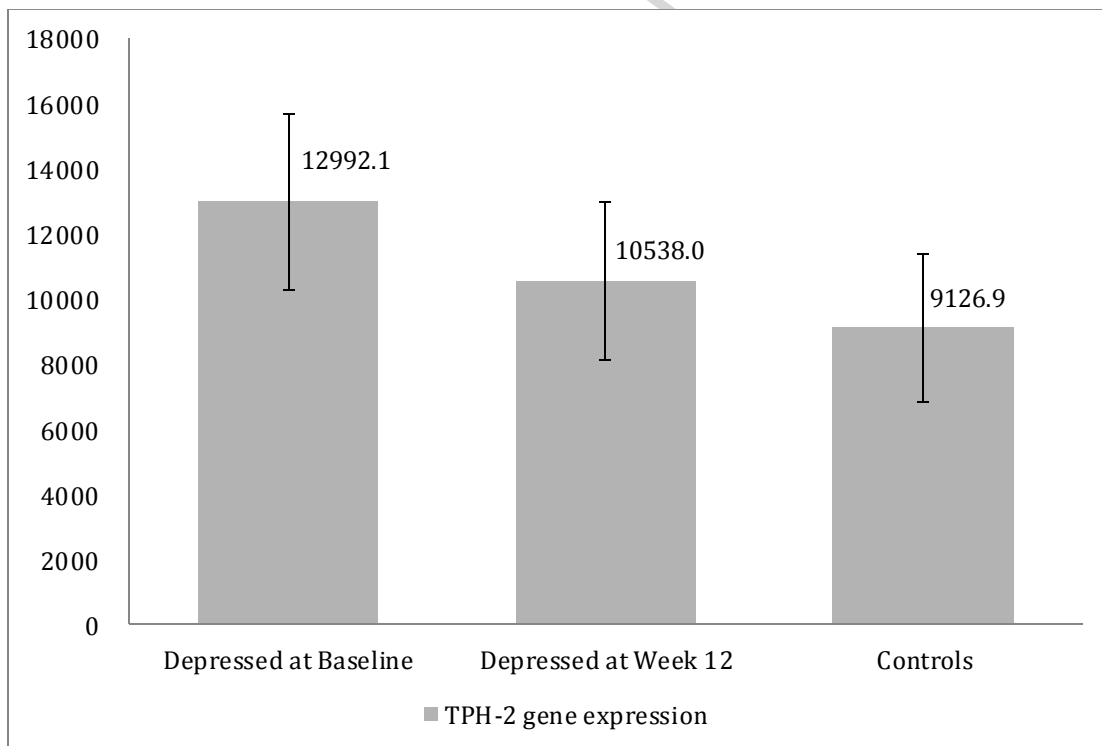
Abbreviations: MDD, major depressive disorder; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HAMD, the 21-item Hamilton Depression Rating Scale

**Legends for Figure 1. The relative gene expressions of PBMCs mRNA of cPLA2 (1a), COX-2 (1b), TPH-2 (1c) and 5-HTT (1d) in healthy controls and major depressive disorders (MDD) patients before and after omega-3 PUFAs treatment.** Results are presented as the mean values in columns and SEM in error bars. Differences between MDD patients and controls were compared by the Mann-Whitney test. There were no significant differences between patients and controls in all four selected gene expressions. Differences between pre- and post-treatment of omega-3 PUFAs in MDD patients were compared by the paired-t test. The levels of relative fold expressions in MDD patients received omega-3 PUFAs did increase in cPLA2 and decrease in COX-2, but not in TPH-2 and 5-HTT genes. Difference was considered statistically significant if a p-value was equal to or smaller than 0.05\*.

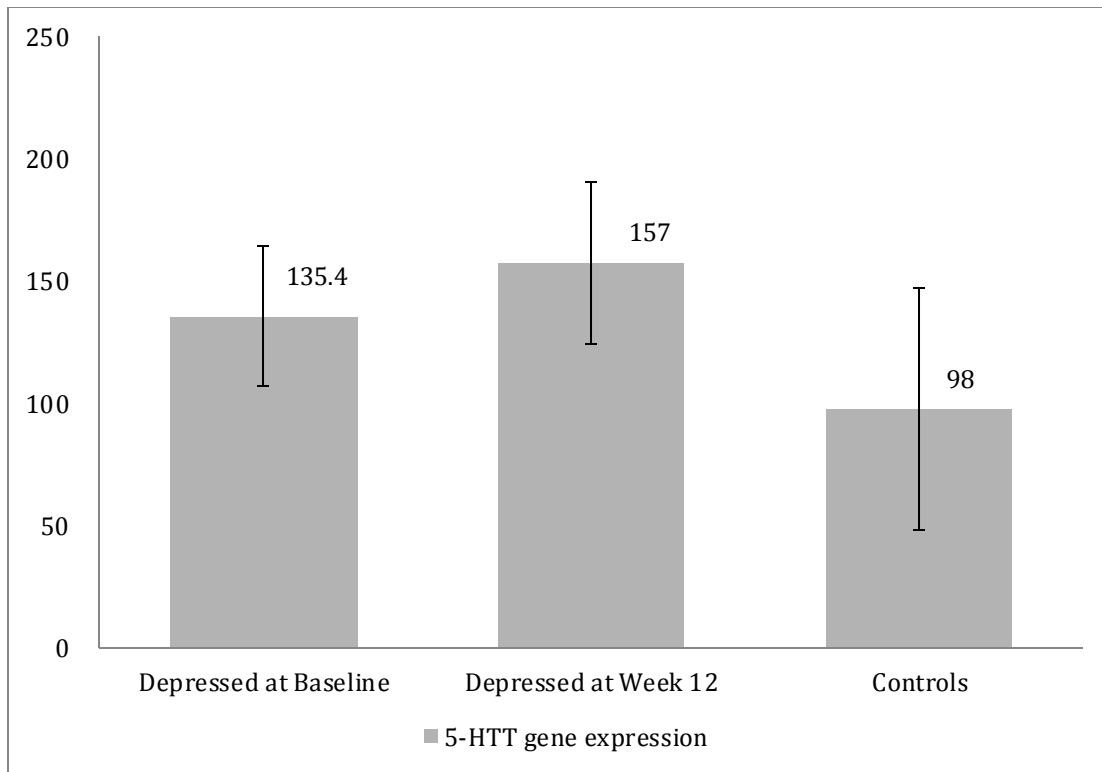




1b



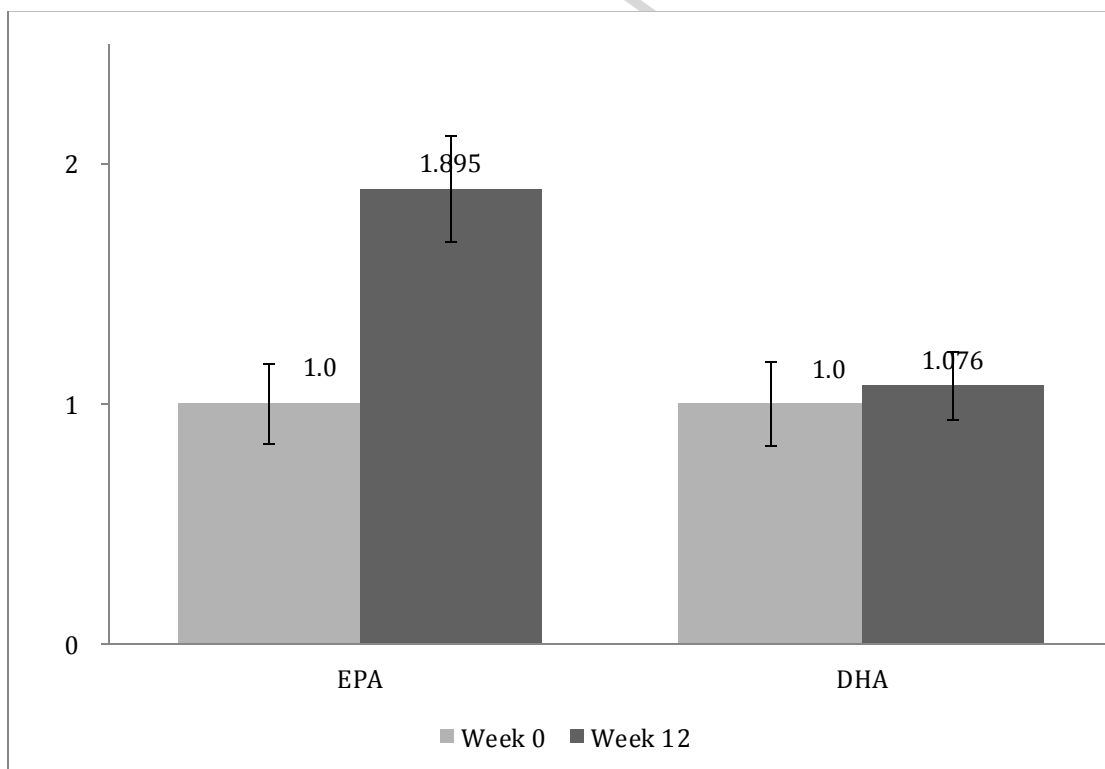
1c



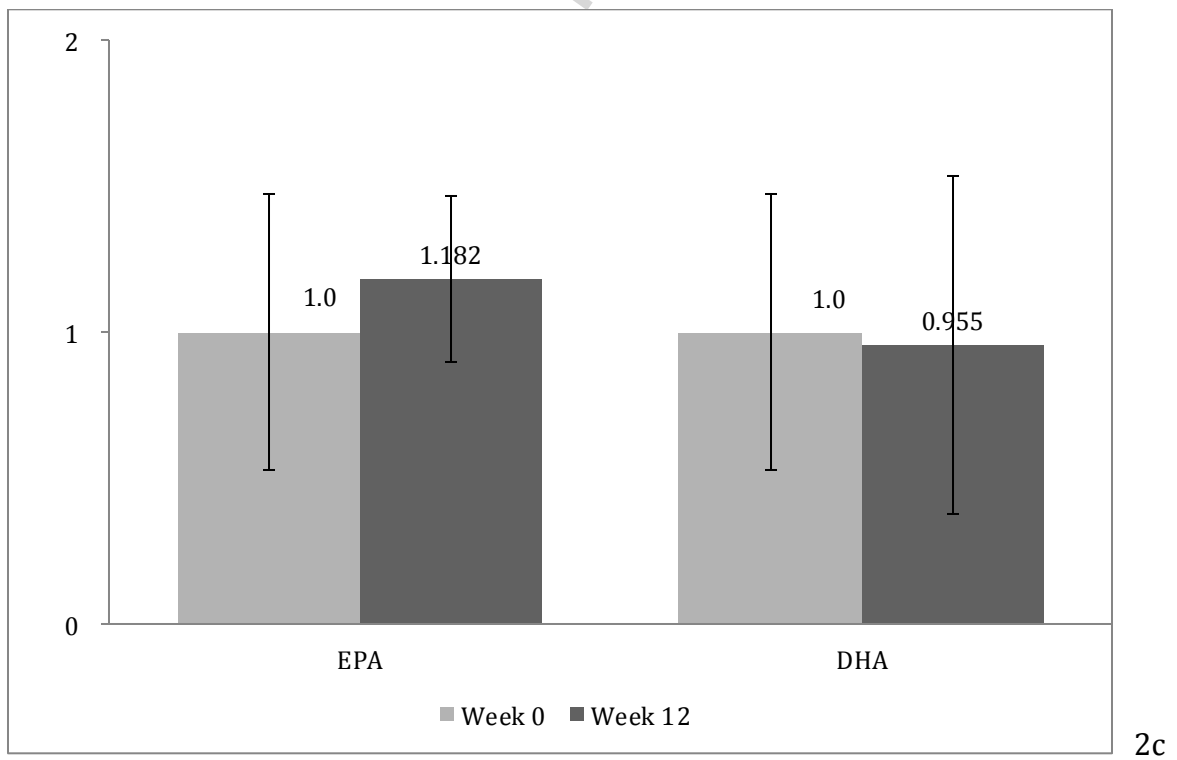
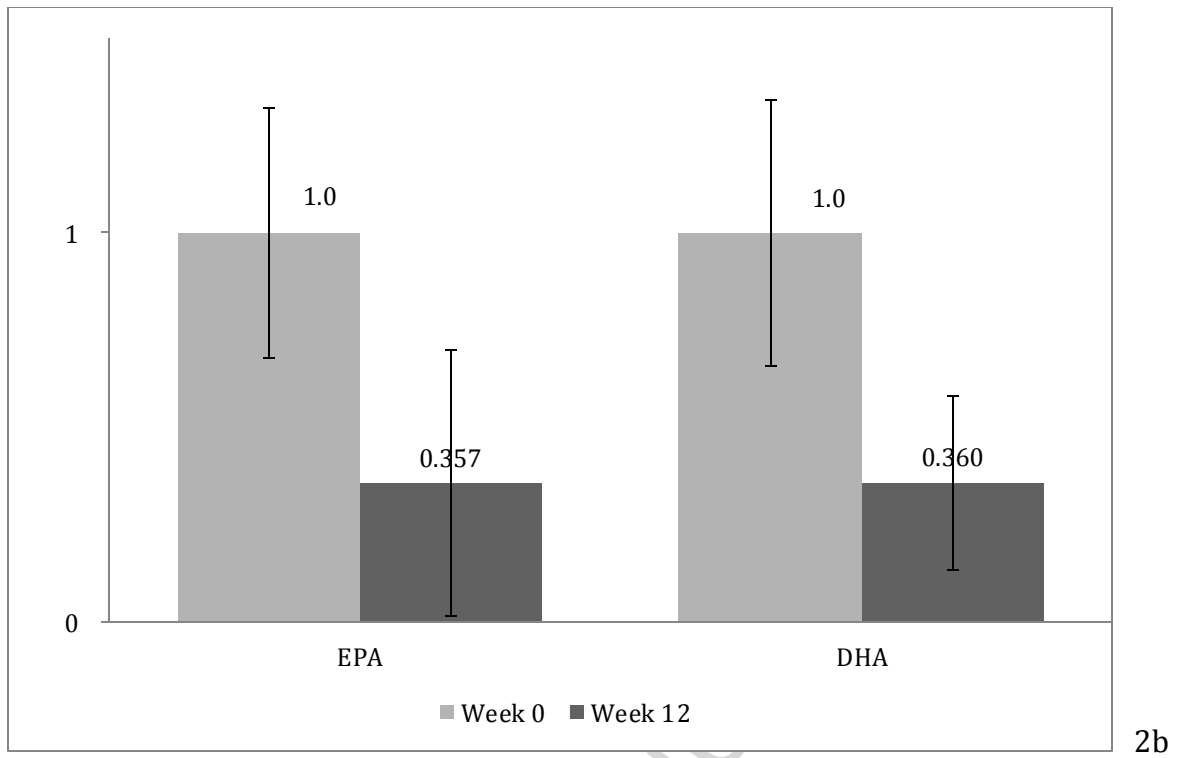
1d

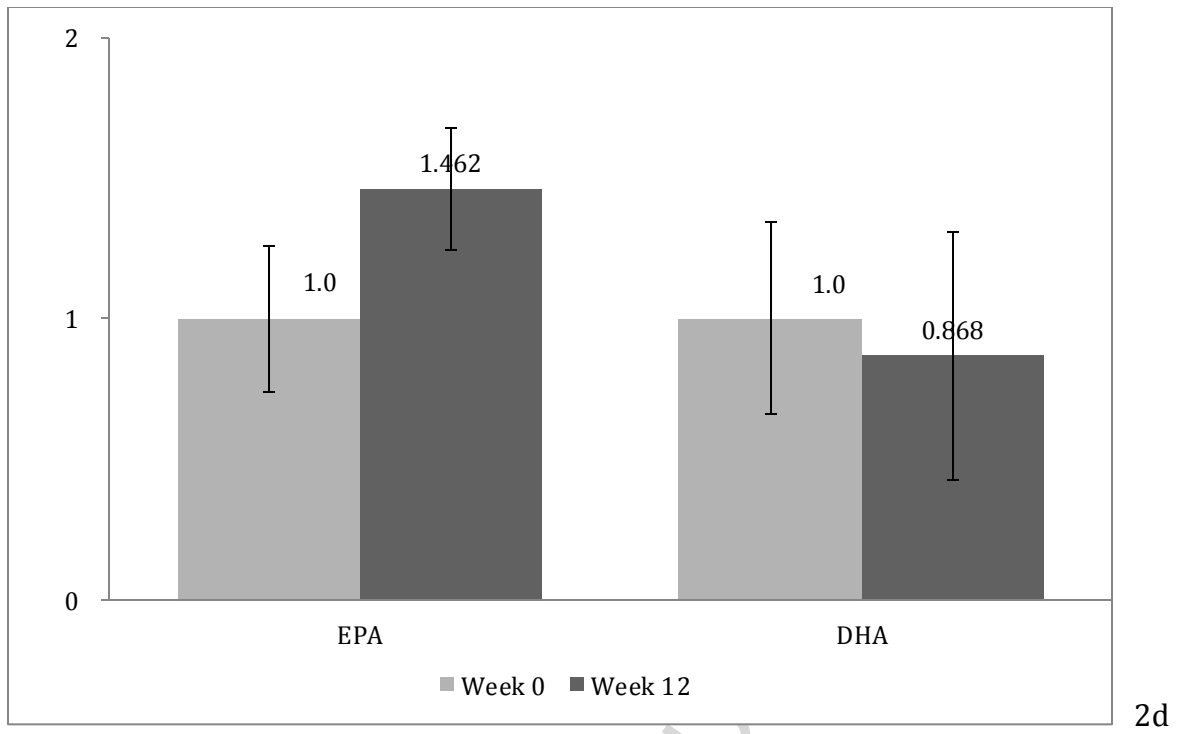
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**Legends for Figure 2. The fold changes of relative gene expression of PBMCs mRNA of cPLA2 (2a), COX-2 (2b), TPH-2 (2c) and 5-HTT (2d) in major depressive disorders (MDD) patients before and after EPA and DHA treatments.** Results are presented as the mean values in columns and SEM in error bars. The baseline values of means of every gene expression are reduced to 1 for reference. The fold changes of mRNA expression after omega-3 PUFAs treatments are presented as ratio as comparing to the baseline respectively., The SEM values are calculated as the ratios according to their mean values. Differences between pre- and post-treatment are compared by paired-t test. MDD patients received EPA treatment did significantly change the gene expressions of cPLA2 (1.89 folds,  $p=0.038$ ), but not COX-2 (0.36 folds,  $p=0.09$ ), TPH-2 (1.18 folds,  $p=1.00$ ) and 5-HTT (1.46 folds,  $p=0.06$ ). DHA treatment did not significantly change of gene expression of any of the 4 genes of cPLA2 (1.08 folds,  $p=0.92$ ), COX-2 (0.36 folds,  $p=0.14$ ), TPH-2 (0.96 folds,  $p=0.62$ ) and 5-HTT (0.87 folds,  $p=0.82$ ).



2a





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