Cognitive vulnerability to depression : genetic and environmental influences

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Chapter 6

Effects of omega-3 fatty acid supplementation on mood and emotional information processing in recovered depressed individuals

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Abstract

Beneficial effects of omega-3 fatty acids have been reported for several psychiatric disorders, but mostly for depression. Association studies show a relationship between omega-3 intake and depression risk. Meta-analyses of clinical trials show a moderate effect of supplementation in depressed patients. Few studies have reported effects on cognition. The purpose of this study was to examine effects of omega-3 supplements on cognition and mood of recovered depressed individuals. Seventy-one participants with at least one episode of depression in the past were randomized to receive either omega-3 or olive oil placebo for 4 weeks in a randomized double-blind design. Results showed an effect of omega-3 supplementation on emotional decision-making and on self-reported states of depression and tension. Participants in the omega-3 group showed decreased recognition of fearful faces compared to placebo, but a learning effect was also evident. Less clear effects were found on the affective attention test and no significant effects were observed on memory, neutral attention, cognitive reactivity and depressive symptomatology. The present findings indicate that omega-3 supplementation had selective effects on emotional cognition and mood in recovered depressed participants. Future studies need to elucidate effects of omega-3 fatty acids in individuals with vulnerability to depression and shed light into plausible neuropsychological mechanisms of action.

Trial registration: ClinicalTrials.gov identifier: NCT01104194

Introduction

Omega-3 (n-3) fatty acids are long-chain polyunsaturated fatty acids (PUFA's) found mainly in fish oil and include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Beneficial effects of n-3 fatty acids have been reported for a range of psychiatric disorders, such as attention deficit hyperactivity disorder, schizophrenia, borderline personality disorder. The most convincing evidence points to efficacy in mood disorders (Ross et al., 2007). N-3 PUFAs may affect neuronal functioning through several mechanisms. These include the decrease of the production of inflammatory eicosanoids from arachidonic acid; inhibition of the release of proinflammatory cytokines; increase of brain-derived neurotrophic factor (Parker, et al., 2006). Furthermore, DHA plays a role in membrane integrity and fluidity (Yehuda et al., 1999). Animal studies have shown that n-3 PUFAs deficiencies may affect dopaminergic and serotonergic neurotransmission (Chalon, 2006; de la Presa Owens & Innis, 1999; Kodas, et al., 2004). In humans, DHA correlated with the concentration of serotonin metabolites in cerebrospinal fluid (Hibbeln, et al., 1998). Reduction of HPA-axis activity is another potential pathway through which omega-3 may exert its effects (Hibbeln et al., 2004; Jazayeri, et al., 2010).

Epidemiological studies show associations between low seafood intake and greater risk of depression with high consistency (Hibbeln, 2009). N-3 fatty acid deficits have been found in red blood cells of patients with Major Depressive Disorder (MDD) (Edwards et al., 1998; Peet et al., 1998). Postmortem studies have found significant DHA deficits in the prefrontal cortex gray matter of patients with MDD (McNamara, et al., 2007). Habitual dietary n-3 fatty acid intake was also positively correlated with gray matter volumes in the amygdala and anterior cingulate in 55 healthy adult individuals (Conklin, et al., 2007). A pilot study showed that DHA was correlated to differential concentrations of regional cerebral glucose metabolism in cortical areas of medication-free depressed patients (Sublette, et al., 2009). McNamara (2010) provide a concise review of the evidence linking (DHA) deficiency and neuropathology in the prefrontal cortex, thus providing another potential mechanism by which n-3 fatty acid deficiency can add to the development of affective dysregulation.

Several meta-analyses have evaluated the effects of n-3 PUFAs on depressed mood from randomized-controlled trials (Appleton et al., 2006; Appleton et al., 2010; Freeman, et al., 2006; Lin & Su, 2007; Ross, et al., 2007). The most recent one shows a

moderate effect of omega-3 supplementation in individuals with a diagnosis of depression (Appleton, et al., 2010). The meta-analysis provides less supportive evidence for beneficial effects in non-psychiatric samples or in patients with other psychiatric diagnoses.

In general, larger effects have been observed in trials where omega-3 was added to anti-depressant ongoing treatment in depressed patients (Nemets et al., 2002; Peet & Horrobin, 2002; Su et al., 2003). However, negative studies also exist (Grenyer, et al., 2007; Silvers et al., 2005). Small effects have been observed in studies examining the efficacy of EPA as a monotherapy (Mischoulon et al., 2009) and no effects were found with DHA alone (Marangell et al., 2003). One study in children with major depression demonstrated a positive effect of omega-3 fatty acid monotherapy (EPA & DHA) compared to placebo (Nemets H et al., 2006). A recent large placebo-controlled clinical trial showed that omega-3 supplementation (high in EPA) reduced depressive symptoms in depressed patients without comorbid anxiety disorders (Lesperance, et al., 2010). Examining another subgroup, the authors found that supplementation had a higher impact as a stand-alone treatment, than among patients taking antidepressants. The effects of supplementation in the whole sample reached only a trend toward superiority of omega-3 over placebo. Overall, the highest efficacy of supplementation is evident in treatments with EPA alone or EPA and DHA combined (with EPA in higher dosages than DHA) (Freeman, et al., 2010).

Most clinical trials have focused on the effects of omega-3 supplementation on mood or symptom improvement. However, subjective improvement of mood may not be always detectable after short-term treatment with omega-3, whereas effects on cognitive functioning may appear. Research on the effects of anti-depressants has shown that cognitive effects may occur independently from symptom changes and that these effects may appear in healthy individuals even after one dosage (Harmer et al., 2009). For instance, a single dose of citalopram improved accuracy and speed of recognition of facial expressions of fear without affecting mood (Browning et al., 2007; Harmer et al., 2003).

Only a few studies have examined effects of omega-3 supplementation on cognition. Fontani et al. (2005) examined the effects of five-week supplementation of omega-3 fatty acids in healthy participants in a placebo-controlled trial and found faster reaction times on attentional tasks. The omega-3 group also reported improved anger, anxiety, fatigue, depression, confusion, and vigor scores. However, we cannot be sure

that the improvements in the n-3 PUFA group were significantly larger than changes in the placebo group, since analysis of variance tests were not conducted and only paired t-tests in the omega-3 group were reported. Furthermore, in a neuro-imaging study, McNamara et al. (2010) found that DHA supplementation for 8 weeks significantly increased prefrontal cortex activation during a sustained attention task in healthy boys, but did not affect behavioral performance on this neutral task (McNamara, et al., 2010). Another trial evaluated the effects of DHA & EPA treatment on cognitive functioning in mild to moderately depressed individuals (Rogers PJ et al., 2008). The test battery in that study was mainly comprised of neutral information processing tasks, with the exception of one affective task, but no differences were found between the omega-3 and placebo groups.

Recently, we explored the effects of omega-3 fatty acid supplements on depression-related cognition and mood in 54 healthy volunteers (Antypa et al., 2009). We examined effects on emotional information processing and we found that the omega-3 group made fewer risk-averse decisions than the placebo group in a gambling task, and these decisions were unrelated to impulsiveness. We also found that omega-3 reduced scores on a control/perfectionism reactivity scale and on self-reported levels of fatigue.

We now aimed to investigate the effects of omega-3 supplementation on the emotional information processing of a more vulnerable group, that of recovered depressed individuals. This is a group susceptible to depressive relapse, often with some residual symptoms of depression. The test battery measured attention to emotional stimuli, response inhibition, facial expression recognition and risky decision-making. We selected a number of cognitive tests, which in previous studies have been shown sensitive to neurotransmitter manipulations (Merens et al., 2007) or to depression vulnerability (Austin et al., 2001). For example, subjects' processing of reward and punishment cues when making risky choices are partly dissociable following manipulations of serotonin (Murphy et al., 2009; Rogers et al., 2003), noradrenaline (Rogers et al., 2004), dopamine (Scarna et al., 2005) and cannabinoid systems (Rogers et al., 2007). We also assessed effects on mood and depressive symptoms. We hypothesized that four weeks of supplementation will positively affect cognitive functions and subjective mood states compared to placebo.

Materials and Methods

Participants

Participants were recruited from June 2007 to July 2009. Eligible participants were fluently Dutch-speaking individuals with age between 18 and 65 years and with a body mass index (BMI) between 18 and 27 kg/m^2. Participants were included if they had a regular diet containing fish not more than once a week and were willing to maintain this diet throughout the study. Participants were eligible if they had a history of at least one major depressive episode in the past. If they were still under treatment, the treatment must have begun more than 3 months prior to study entry, and medication dosage and/or frequency of therapeutic sessions must remain stable. Exclusion criteria were: Beck Depression Inventory-II-NL score higher than 19, or score on the suicidality item of this scale higher than 1, current or past psychosis, current substance abuse or past substance dependence, smoking or current use of soft drugs (month prior to study entry), hard drug use (lifetime), and more than three alcoholic consumptions/day, current use of omega-3 supplements, current diagnosis of attention-deficit hyperactivity disorder.

Intervention and Blinding

Participants took 3 grams of fish oil or olive oil (placebo) per day, provided in three soft-gel capsules, for four weeks. The fish oil contained 2.3g of n-3 PUFA (1.74g EPA, 0.25g DHA). The dose and duration were chosen on the basis of a consensus paper (Freeman et al, 2006). Both the fish-oil and placebo capsules were lemon-flavoured to maintain the blind. Self-reported compliance and the success of blinding were checked by a questionnaire after the completion of the study. This was a double-blind randomized controlled trial with research assistants, psychologists and participants being blind to group assignment. Randomization was carried out in blocks of six. An independent person, who had no access to participants' names and data collected, allocated participant codes to the numbers of the randomization list. The experimenters received the box of capsules (which was identical for both groups) the same day that the participant started the first session. The study was approved by the Medical Ethics Committee of Leiden University Medical Center, in The Netherlands.

Instruments

The expanded version of the Mini-International Neuropsychiatric Interview (M.I.N.I. PLUS) version 5.0.0. was used to assess current and lifetime DSM-IV diagnoses (Sheehan & Lecrubier, 1998; 2006).

Outcome Measures

The primary outcome measures were cognitive performance tests.

Neutral and Emotional Information Processing tasks

An *Affective Go/No-Go task* was used, consisting of eight blocks of 16 stimuli. In each block, single words were presented with a stimulus interval of 1200ms. Half of the stimuli were positive words (e.g. happy, clean) and half were negative (e.g. sad, guilt). In each block, either the positive (P) or negative (N) words were designated 'targets'. The blocks were presented in a NNPPNNPP or PPNNPPNN order. Due to this arrangement, three blocks were `shift ' blocks, where participants had to withhold responding to stimuli that were targets in the previous block, and four blocks were `non-shift' blocks.

An Attentional Go/No-Go task was also used, with parameters identical to the *Affective Go/No-Go task*, except that letters and digits were used as targets.

The Facial Expression Recognition Task featured five basic emotions (happiness, sadness, fear, anger and disgust) taken from the Pictures of Facial Affect Series (Ekman & Friesen, 1976). A male and female example of these pictures was morphed between each prototype and neutral in 10% steps. Four trials of each emotion were presented at each intensity level. These stimuli were presented in randomized order for 500 ms and replaced by a blank screen. Participants were asked to respond as quickly and accurately as possible.

The *15 words test* was used to assess memory performance (Saan & Deelman, 1986). A list of 15 unrelated, neutral words was presented from tape. Immediate recall was tested after each of two consecutive presentations. Participants continued with non-verbal tasks and after twenty minutes delayed recall was tested. Parallel versions were used at each session in a randomized sequence. "Immediate recall" was defined as the total number of correct words from the first and second trial; "delayed recall" was defined as the total number of correct words from the recall after the twenty minutes delay.

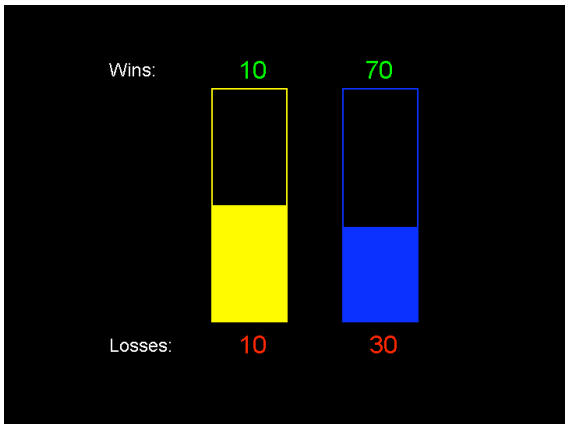
The *Decision-making (gambling) task* measures decision making behaviour over a variety of differentially weighted contingencies and also distinguishes risk-seeking from risk-aversive behaviour. It has been previously described in detail by Rogers et al. (2003). On each trial, participants were asked to choose between two simultaneously presented gambles. Each gamble was visually represented by a histogram, the height of which indicated the probability of gaining a given number of points (see Figure 1A). The possible gains were indicated in green ink above the histogram and the potential losses were indicated in red ink below the histogram. One gamble (coloured yellow) was always the control gamble, which had a 0.50 probability of winning 10 points and a 0.50 chance of losing 10 points (with an expected value of 0). The alternative 'experimental' gamble (coloured blue) varied in the probability of winning which was either high or low (0.60 vs. 0.40), possible gains which were either large or small (70 vs. 30 points) and possible losses which were either large or small (70 vs. 30 points). These variables were crossed to produce eight trial types in which the expected value of the 'experimental' gamble varied between -20 and + 20. The control and the 'experimental' gamble appeared randomly on the left or right. The participants indicated their choice by a key press. Dependent measures were the proportion of choices of the 'experimental' over the control gamble as a function of its probability of winning, size of possible gains and the size of possible losses, and the mean deliberation time (ms) for these choices.

Two extra trial types were also included that represented choices between gambles known to be subject to the non-normative biases of risk-aversion and risk-seeking choices and that cannot be explained by decision-makers choosing actions that maximise expected value (the 'reflection effect'; see Kahneman and Tversky, 1979). The first such trial type was a 'gains-only' trial in which the participants were presented with a choice between a guaranteed win of 30 points vs. a 0.5 chance of winning 60 points and a 0.5 chance of losing 0 points (see Figure 1B). Neither option had any associated losses. By contrast, the second trial type was a 'losses-only' trial in which the participants were presented with a choice between a guaranteed loss of 30 points vs. a 0.5 chance of losing 60 points and a 0.5 chance of losing 0 points. Neither option had any potential gains. Within both the 'gains-only' and 'losses-only' trial types, the expected value of each gamble was equal; however, decision-makers usually exhibit risk-aversion in the former case but risk-seeking behaviour in the latter case (Schneider & Lopes, 1986).

The ten trial types were presented pseudorandomly within 4 blocks of 20 trials. Across the 4 blocks, there were 8 repetitions of each 'experimental' gamble and 8 repetitions of

Figure 1. An example visual display from the decision-making task, consisting of an 'experimental' gamble with a 0.40 chance of winning 70 points and a 0.60 probability of losing 30 points versus the control gamble with a 0.50 chance of winning 10 points and a 0.50 of losing 10 points **(Panel A)**. A 'Gains only' trial consisting of a certain win of 30 points and a gamble with a 0.50 probability of winning 60 points or 0 points **(Panel B)**. A 'Losses only' trial consisting of a certain loss of 30 points and a gamble with a 0.50 probability of losing of 60 points or 0 points **(Panel C)**.

A



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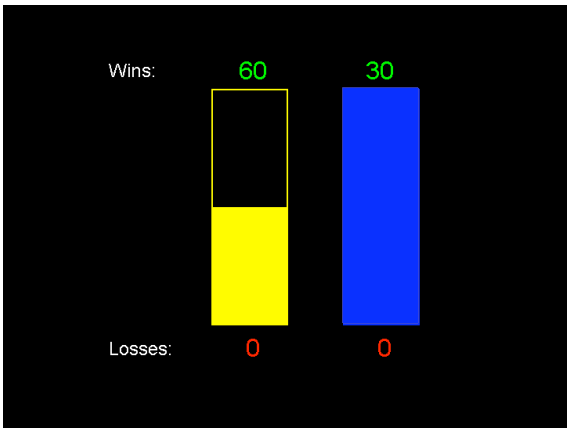

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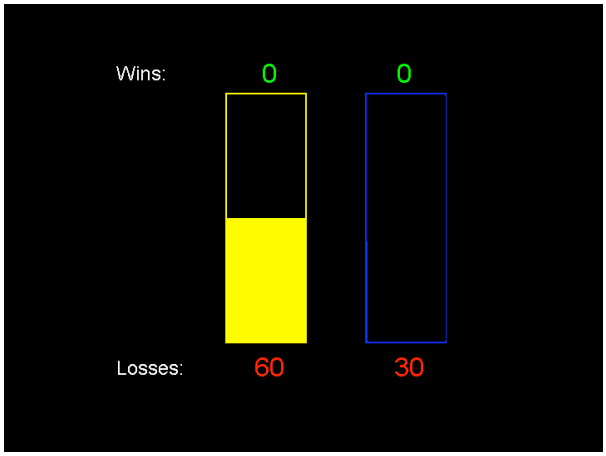


each of the 'gains-only' and the 'losses-only' trial types. In order to maintain participants' interest in the test, a monetary reward of 1 eurocent was placed on each point. Participants started off each block of trials with 100 cents and could keep the average gain of all the blocks (total gains were typically around 3 euros). Visual feedback was given after each choice and the revised points total was presented for 2 s before the next trial. At the end of each block, the participants were given a final score for that block. This task was administered only at post-treatment, as previous pharmacological studies of decision-making involving within-subject designs have found significant order effects (Wood et al., 2006).

Self-report measures

The Beck Depression Inventory - II (BDI - II; Beck et al. 1988; Van der Does, 2002a) was used to measure symptoms of depression during the past two weeks. Mood states during the past week were measured with the shortened Profile of Mood States (POMS) (McNair et al., 1971; Wald & Mellenbergh, 1990). The subscales are depression, anger, fatigue, tension and vigour. Finally, cognitive reactivity was measured with the Leiden Index of Depression Sensitivity - Revised (LEIDS-R; Van der Does, 2002b; Van der Does & Williams, 2003). The subscales are Hopelessness/Suicidality (HOP); Acceptance/Coping (ACC); Aggression (AGG); Control/Perfectionism (CTR); Risk Aversion (RAV); Rumination (RUM). The scale has been found to discriminate between never-depressed and recovered-depressed individuals (Van der Does, 2002b; Merens, et al., 2005) and predicts response to serotonin challenge (Booij & Van der Does, 2007).

Procedure

Participants were recruited through advertisements in university buildings and in the local and university newspapers. Potential eligible participants were first screened via telephone/email. If participants did not fulfil any exclusion criterion, they were invited for a longer in-person screening session, which started with the informed consent procedure, followed by the M.I.N.I.-PLUS and a check of the other in- and exclusion criteria as well as a review of the individual's psychiatric treatment history. In a subsequent session a few days later a blood sample was taken at the Leiden University Medical Center. Subsequently, the first assessment session took place at the laboratories of the Psychology Department of Leiden University, where the questionnaires and tests were administered (t_0) and the intake of supplements started the same day. The cognitive

tests and the POMS were re-administered 1 week later (t_1), and 4 weeks later the same procedure was repeated as at pre-treatment with the addition of the decision making task (t_4). All measurements were taken at the same time of the day for each participant. Participants received 45 euros or course credit.

Statistical analysis

Our analyses followed the intent-to-treat principle. We used SPSS version 17.0. The questionnaires and the cognitive tasks were analysed using repeated measures analysis of variance (rmANOVA), with treatment group (n-3 PUFAs vs. placebo) as between-subjects factor and time as within-subjects factor. Data from the Affective Go/No-Go task were analysed with additional within-subject factors target valence and shift condition. Dependent measures for both Go/No-Go tasks were omissions (failures to respond to targets), false alarms (response to non-targets) and reaction times for hits. For the emotion recognition task, total accuracy scores for each emotion were calculated by adding all the 10% intensity blocks (total possible score: 40) and were analyzed with time as within-subject factor. Accuracy data for each emotion at 20% intensity levels were analyzed with rmANOVA with time and intensity as within-subject factors. Reaction times were examined with time as a within-subject factor. The 15-words list task had immediate recall and delayed recall as outcome measures with time as within-subject factor. For the decision-making task, the proportionate choice data and mean deliberation times were analyzed with rmANOVAs with between-subject factor of treatment, and within-subject factors of probability of winning, size of possible gains and size of possible losses. The 'gains-only' and 'losses-only' trials were analyzed with rmANOVA with between-subject factor of treatment, and within-subject factor of trial type. Cohen's *d* effect sizes were computed by determining differences between omega-3 and placebo mean pre-post changes (Δmean n-3)−(Δmean placebo) divided by their pooled standard deviation (cf. Lucas, Asselin, Merette, Poulin, & Dodin, 2009).

Results

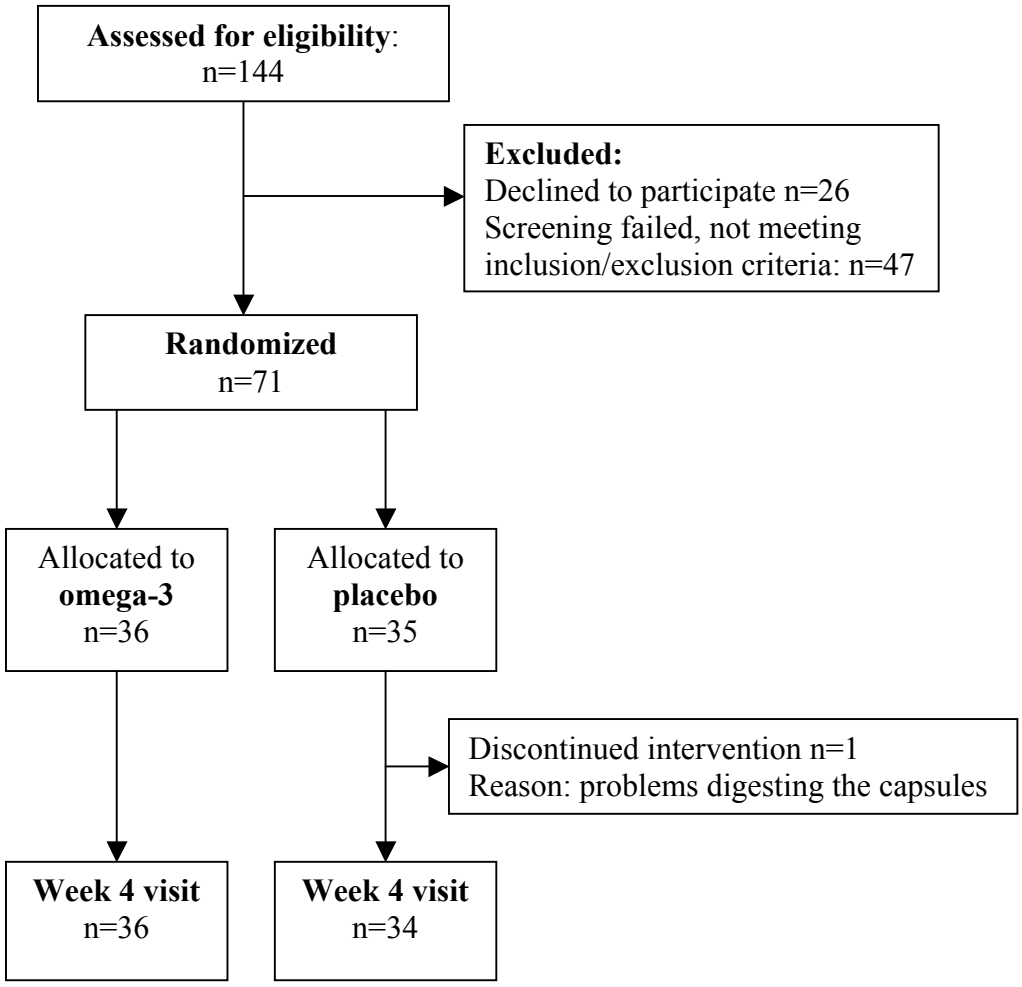
Data Screening

Prior to analysis, all data were examined for accuracy of data-entry, missing values, normal distribution assumptions, and outliers. Extreme outlying data ($z > |3.2|$) were improved by transformations. Scores were normally distributed after square root transformations were performed on the POMS anger, tension, depression and fatigue subscales and on BDI total scores. Logarithmic transformations were performed on the ASI total scores. The LEIDS-R total scores and subscales as well as the BIS/BAS scores were normally distributed with no outliers. In the attentional and affective go-nogo task, logarithmic transformations were performed on misses and false alarms. In the Go-NoGo tasks, when a participant missed 8 trials (1 block), this number was replaced with the mean number of omissions of the group. Reaction times were square root transformed in the *affective Go/No-Go task*. For the decision-making task, proportionate choice data were arcsine-transformed. Tables show untransformed values.

Participant Flow and Characteristics

Of the 144 individuals who showed interest in participating in the study, 71 participants fulfilled the inclusion criteria and were randomized to receive omega-3 (n=36) or placebo (n=35) (see Figure 2). Seventy of the 71 included participants completed the study. The participant that dropped-out had been allocated to the placebo group, and discontinued the study right after the second measurement, due to problems with digesting the capsules. The last available scores of this participant were used as post-test scores for the intent-to-treat analysis.

Figure 2. Participant flow diagram of progress through the trial.



This was a young sample, comprised of predominantly female participants, with an early age of depression onset (17 years). Both groups had an average of three to five episodes of depression in the past. At baseline, there were no differences between the treatment groups on demographic and lifestyle characteristics. There were also no between group differences in the number of comorbid disorders, current and past treatments and symptom severity of previous depression. We also compared the two groups on baseline POMS, BDI-II and LEIDS-R scores. The omega-3 group reported significantly lower levels of lack of vigour compared to the placebo group; no differences were found on the other measures. CR scores in the whole sample were indicative of depression vulnerability as they were higher than those reported by healthy people (Antypa et al., 2009) effect size > 1. The participant characteristics of the omega-3 and placebo groups are shown in table 1.

Table 1. Baseline Characteristics of Participants (n=71) [1]

	Treatment condition		
	Omega-3 (n=36)	Placebo (n=35)	*P* value [2]
Gender –Females	83.3 %	80 %	.72
Age (years)	25.8 ± 11.8	23.5 ± 6.0	.30
In a Relationship	41.6%	57.1%	.19
Education (university)	88.8%	94.2%	.71
Employed	61.1%	68.6%	.51
Vitamin supplements	19.4%	40%	.06
BMI (kg/m^2)	21.5 ± 2.0	21.6 ± 2.0	.84
Psychiatric Diagnoses:			
Past Major Depression (n)	36	35	
Age of onset	17.3 ± 7.7	17.0 ± 5.6	.88
Number of episodes	3.0 ± 2.3	5.2 ± 12.5	.32
Comorbidity (n)			
No comorbid diagnosis(CD) (n)	24	22	
Number of CD	1st 2nd 3rd	1st 2nd 3rd	1st 2nd 3rd
- Dysthymia	3	5	
- Hypomanic Episode	3	5^ 1	
- Panic Disorder	3 1	1 1	
- Agoraphobia	2^	1	
- Past Anorexia Nervosa	2		
- Bulimia Nervosa	1^		.42 .41 .39
- Child ADHD		1 1	
- Social Anxiety		1 1	
- Post-traumatic Stress Disorder		1	
- Premenstrual Syndrome	1^		
- Past alcohol abuse		1	
Treatment	Past Current	Past Current	Past Current
Psychotherapy	12 2	15 4	
Anti-depressants	1 3	3 2	
Combination	8 2	6 -	.56 .42
Light Therapy	1 -	- -	
No Therapy	14 29	11 29	
Characteristics of Past Depressive Episode			
Past diagnostic criteria (total)	6.9 ± 1.4	7.1 ± 1.6	.58

- Weight change (n)	13	17	.29
- Sleep problems (n)	24	26	.48
- Psychomotor agitation (n)	19	20	.71
- Fatigue (n)	32	33	.41
- Worthlessness/Guilt (n)	34	29	.12
- Concentration problems (n)	32	28	.30
- Suicidal Ideation (n)	24	19	.29
Depressive Symptoms (BDI-II)	5.7 ± 5.0	7.7 ± 5.9	.13
LEIDS-R baseline	46.0 ± 16.4	49.7 ± 14.9	.33
POMS baseline			
Fatigue	4.8 ± 4.1	4.2 ±3.2	.53
Anger	2.4 ± 2.2	3.8 ± 3.7	.06
Tension	3.1 ± 2.5	3.7 ± 3.0	.39
Sadness	3.1 ± 4.6	3.6 ± 3.6	.66
Loss of Vigor	9.0 ± 4.0	11.5 ± 4.0	.01

[1]Values display means ± standard deviations, or percentages (%), or number of participants (n) in each group, depending on the distribution.

[2] P values were obtained by independent sample t-tests for continuous variables and chi-square tests for categorical variables.

^ one participant had a current diagnosis

Compliance and Manipulation Check

Self-reported compliance was excellent (99% for the omega-3 group and 97% for the placebo group) and there was no difference between the two groups [$t(69) = 1.5$; $p = 0.14$]. The blinding of participants toward treatment allocation was successful. Nineteen out of 36 (53%) participants in the omega-3 group and 16 participants out of 35 (46%) in the placebo group correctly guessed their group allocation. Ten (28%) participants in the n-3 PUFA group and seven (20%) participants in the placebo group guessed wrongly. The participants of the omega-3 group were in average 59% certain of their guess, and participants in the placebo group were 63% certain of their guess. Nineteen participants (27% of the whole sample) had no idea about treatment assignment. The omega-3 group had a higher percentage of correct and wrong guesses, whereas the placebo group had a higher percentage of participants with no idea of group allocation ($\chi^2(2) = 8.2$; $p = 0.02$).

Self-report measures

Mood states: Separate rmANOVAs on each subscale showed a significant time x treatment interaction on depression [F(1,69)=4.75, p=0.03] and on tension [F (1,69) = 4.59, p=0.04]. The same interaction reached significance at trend level on the fatigue subscale [F(1,69)= 3.61, p=0.06]. There were no significant time x treatment interactions on anger and lack of vigour (p>0.10). We further examined between-group comparisons at separate time points for each of the significant interactions. No differences were found for depression and tension at t_0 ($p > 0.10$); a significant difference after four weeks of omega-3 supplementation was found on depression [t(69)=2.85, p=0.006) and on tension [t (69)=2.45, p=0.02]. Mean scores are presented in table 2. On depression, the effect size was d=0.47, on tension d=0.48, and on fatigue d=0.46.

No significant time x treatment interactions were found on LEIDS-R total, on its subscales and on the BDI-II (see table 2).

Table 2. Mood states, cognitive reactivity and depressive symptoms before and after supplementation.

	Omega-3 (n = 36)		Placebo (n = 35)		Time x treatment interaction (p value)
	Pre-	Post	Pre-	Post	
POMS					
Depression	3.1 ± 4.6	2.3 ± 4.4	3.6 ± 3.7	4.8 ± 4.8	P = 0.03
Tension	3.1 ± 2.5	2.5 ± 3.0	3.7 ± 3.0	4.4 ± 3.7	P = 0.04
Fatigue	4.8 ± 4.1	3.8 ± 4.5	4.2 ± 3.2	5.1 ± 4.6	P = 0.06
Anger	2.4 ± 2.2	2.4 ± 2.4	3.8 ± 3.7	3.8 ± 3.7	P > 0.10
Lack of vigour	9.0 ± 4.0	9.3 ± 4.4	11.5 ± 4.0	11.2 ± 3.7	P > 0.10
LEIDS-R total	46.0 ± 16.4	43.9 ± 13.7	49.7 ± 14.9	49.3 ± 16.2	P > 0.10
BDI Total	5.7 ± 5.0	6.6 ± 7.3	7.7 ± 5.9	6.5 ± 6.4	P > 0.10

Emotional and Neutral Information Processing

Attentional Go/No-Go task

Analyses revealed no significant time x treatment interaction on the number of omissions [F(2,138)=0.81,p=0.45], the number of false alarms [F(2,138)=0.02, p=0.99] and on reaction times [F(2,138)=0.71, p=0.49]. (Table 3 displays means and standard deviations).

Affective Go/No-Go task

Omissions: Overall, participants had the highest mean number of misses in shift blocks with positive targets and the lowest mean number of misses in shift blocks with negative targets (shift*valence interaction, $p<0.05$). We found no significant time x valence x shift condition x treatment interaction [F(2,138)=0.79, p=0.46]. Three-way interactions were also non-significant ($p>0.90$). We found time x treatment interaction significant at trend level [F(2,138)=2.56, p=0.08], yielding a marginally significant quadratic contrast [F(1,69)=3.85, p=0.05]. Since valence and shift conditions did not interact with treatment group, we further examined the total number of misses collapsed across conditions. A rmANOVA showed a marginally significant time x treatment interaction on the total number of misses [F(1.6,110.6)=3.22, p=0.05] yielding a significant quadratic contrast [F(1,69)=4.08, p=0.047] (*d*=0.31) (see Table 3). Independent sample t-tests for separate time points showed that the two treatment groups did not differ (only at t_1, p=0.07).

False commissions (false alarms): Overall, participants made had a higher false alarm rate in shift blocks compared to non-shift blocks ($p<0.05$), but no differences were found between positive and negative blocks. The time x shift x valence x treatment interaction was significant at trend level [F(2,138)=2.64, p=0.075], yielding a significant linear contrast [F(1,69)=4.20, p=0.04]. Furthermore, the linear contrast of the time x shift x treatment interaction was marginally significant [F(1,69)=3.93, p=0.05]. We explored these interactions by conducting separate rmANOVA's for shift blocks and non-shift blocks with valence and time as within subject factors and then followed significant interactions. In *shift blocks with negative targets*, we found a significant time x treatment interaction [F(2,138)=4.71, p=0.01] but this interaction was mainly driven by the placebo

group showing a decrease in false alarms after supplementation, whereas the omega-3 group showed small pre-post change (*d*=-0.86)(see Table 3). In *shift blocks with positive targets*, the time x treatment interaction was not significant [F(2,138)=0.03, p=0.97]. In *non-shift blocks*, we found a non-significant time x valence x treatment interaction [F(2,138)=1.07, p=0.34].

Reaction times: A rmANOVA on reaction times showed a non-significant time x shift x valence x treatment interaction [F(2,138)=1.16, p=0.32]. The three-way time x shift x treatment interaction yielded a significant linear contrast [F(1,69)=5.34, p=0.02]. No other three- and two- way interactions were significant. Subsequently, reaction times were collapsed for valence to examine shift and non-shift conditions separately. In *shift blocks*, we found a significant time x treatment interaction [linear contrast: F(1,69)=4.70, p=0.03], where the omega-3 group had faster responses over time compared to placebo (*d*=0.31; see Table 3). Independent sample t-tests showed that the two groups did not significantly differ at any time point. In *non-shift blocks*, no significant interaction was found (p=0.92).

15 word list (memory) test

We found no significant time x treatment interactions on immediate recall [F(2,138)=0.4, p=0.67], and delayed recall [F(2,138)=0.36, p=0.70] (see Table 3 for means and standard deviations).

Facial Emotion Recognition

A repeated measures with total emotion accuracy (collapsed across intensities) showed a main effect of time [F(1.6, 114.7)=74.9, p< 0.001] and a main effect of emotion [F(3.1, 214.4)=59.7, p < 0.001], indicating that participants improved over time and were better at recognizing certain emotions compared to others. The time x emotion x treatment interaction was significant at trend level [F(5.6, 383.7)=1.81, p=0.10] but yielded a significant linear contrast [F(1,69)=4.17, p=0.045]. Analysis with total accuracy scores for each emotion separately showed a significant time x treatment interaction on fear [F(1.68, 116.07)=4.29, p=0.02]. The omega-3 group showed a lower rate of correct recognition of the fear emotion over time (*d*=0.50), but at post-test we found no significant difference between omega-3 group and placebo (p=0.34). The time x treatment interactions on anger, disgust, happy, and sad emotions were not significant

Table 3. Means (±Standard deviations) of performance on the *attentional* and *affective Go/No-Go tasks*, on the 15-word test (memory) and on facial emotion recognition.

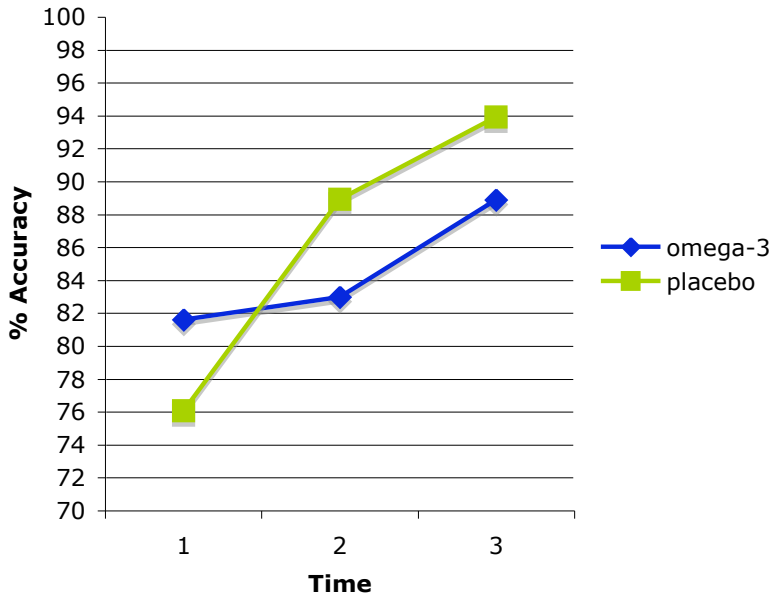
	Omega-3		Placebo		Time x Treatment interaction *(P value)*
	Pre	Post	Pre	Post	
Attentional Go/no-go					
Omissions	2.4 ± 3.0	1.7 ± 3.4	3.4 ± 4.9	1.0 ± 2.0	.45
False alarms	4.2 ± 3.4	1.5 ± 1.8	4.1 ± 4.8	1.3 ± 1.8	.99
Reaction Time	406.2 ± 43.9	413.4 ± 50.1	399.6 ± 40.7	414.9 ± 46.8	.49
Affective Go/no-go					
Omissions (Total)	0.48 ± 0.65	0.27 ± 0.44	0.47 ± 0.63	0.54 ± 0.92	.047* [a]
False alarms					
Shift blocks					
-Negative	0.25 ± 0.50	0.39 ± 0.60	0.83 ± 1.0	0.29 ± 0.52	.01* [b]
-Positive	0.58 ± 0.81	0.33 ± 0.59	0.77 ± 0.97	0.49 ± 0.70	.97
Non-shift blocks					
-Negative	0.44 ±0.84	0.22 ± 0.54	0.37 ± 0.69	0.40 ± 0.65	.34
-Positive	0.47 ± 0.70	0.25 ± 0.60	0.51 ± 0.91	0.26 ± 0.50	
Reaction times					
-Shift blocks	604 ± 92.4	581 ± 65.1	584 ± 69.1	596 ± 76.7	.03* [c]
-Non-shift blocks	586 ± 65.4	598 ± 69.7	593 ± 75.6	601 ± 91.8	.92
15 word list (memory)					
Immediate recall	17.6 ± 4.4	19.8 ± 4.5	18.1 ± 3.9	19.4 ± 4.2	.67
Delayed recall	9.0 ± 2.5	8.5 ± 3.1	8.7 ± 2.4	8.4 ± 2.9	.70
Facial Expression Recognition Test					
Accuracy (total)					
Anger	21.0 ± 3.7	24.3 ± 3.5	21.1 ± 3.4	24.0 ± 4.7	.82
Fear	23.5 ± 4.7	24.8 ± 3.4	22.5 ± 4.8	25.7 ± 5.0	.02* [d]
Disgust	25.6 ± 4.2	27.9 ± 3.8	26.5 ± 4.9	28.1 ± 3.6	.57
Happy	27.8 ± 3.3	29.4 ± 2.9	27.9 ± 3.6	30.2 ± 3.8	.59
Sad	18.3 ± 6.8	22.5 ± 6.9	17.5 ± 7.7	24.1 ± 8.6	.22
Reaction Time					
Anger	1082 ± 299	880 ± 257	959 ± 278	823 ± 206	.10
Fear	1079 ± 415	897 ± 302	942 ± 261	750 ± 215	.25
Disgust	898 ± 267	688 ± 221	773 ± 215	632 ± 168	.19
Happy	778 ± 201	626 ± 179	732 ± 158	603 ± 174	.71
Sad	1095 ± 238	831 ± 294	1045 ± 332	787 ± 229	.71

*Independent sample t-test between omega-3 and placebo at post-test was not significant.
Effect sizes: [a] d=0.31, [b] d= -0.86, [c] d=0.31, [d] d=0.50

(all p's > .05). A main effect of time was found on the accuracy scores of all emotions. (see Table 3 for means and standard deviations of accuracy scores on all emotions).

Separate analyses per emotion, with emotion intensity as a within subject factor, showed a significant time x treatment interaction only on fear recognition [F(2,138)=4.29, p=0.02]. A significant time x intensity x treatment quadratic-linear contrast was also found [F(1,69)=6.28, p=0.015]. We then examined the time x treatment interaction with rmANOVAs on fear accuracy for each intensity level (five 20% blocks) separately. We found a significant interaction in the 50-60% intensity level [F(2,138)=3.65, p=0.03]: the omega-3 group had a lower rate of accurately recognizing fear over time (d=0.46) (see figure 3). A significant time x treatment interaction was found also at the 100% intensity level, F(2,138)=4.53, p=0.01. Visual inspection of the graph showed that this interaction was driven by baseline differences, and that the omega-3 group showed almost no pre-post change.

Figure 3. Recognition of fear at 50%-60% intensity across time (time x treatment interaction, p=0.03, effect size: d=0.46)



Decision Making *(n=70)*

Proportionate Choice & Deliberation Times

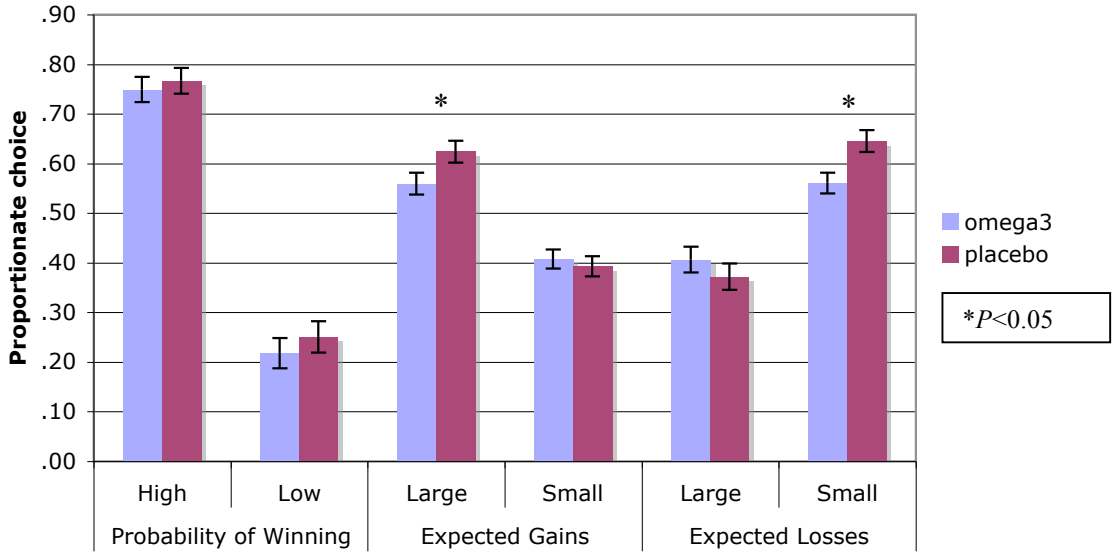
Participants chose the experimental gamble significantly more often when the probability of winning was high compared to when it was low [$F(1,68) = 262.2$, $p < 0.001$] and treatment did not significantly affect this choice ($p=0.81$). Similarly, participants chose the experimental gamble significantly more often when the expected gains were large compared to when they were small [$F(1,68) = 139.6$, $p < 0.001$], however, a significant two-way interaction between size of gains and treatment was found [$F(1,68)=6.08$, $p=0.016$]. Additional analysis showed that when gains were large, the omega-3 group chose the experimental gamble significantly less often than the placebo group [$F(1,68)=4.34$, $p=0.04$]; when gains were small, there were no differences in the choices between the groups [$F(1,68)=0.28$, $p=0.6$] (see Figure 4a).

Participants chose the experimental gamble less often when expected losses were large compared to when they were small [$F(1,68) = 83.8$, $p < 0.001$], but treatment modified this choice ($F(1,68)=6.44$, $p = 0.01$). Additional analysis showed that when losses were small, the omega-3 group chose the experimental gamble significantly less often than the placebo group [$F(1,68)=7.72$, $p=0.007$]; when losses were large, there was no difference between the two groups in their choice of the experimental gamble (see figure 4a).

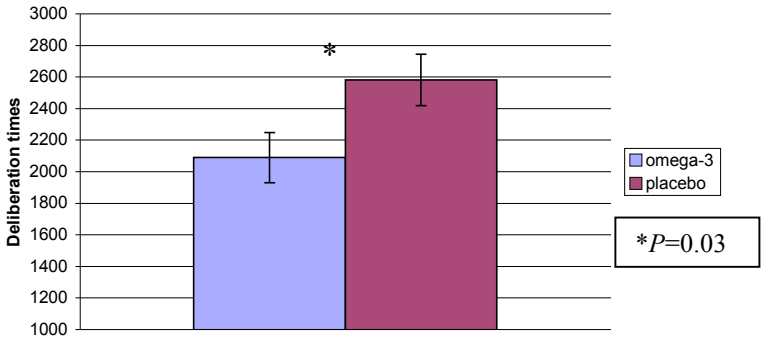
There were no significant three-way interactions between treatment group, probability and size of gains or losses ($p > 0.05$). However, the four-way probability x gains x losses x treatment interaction was significant [$F(1,68)=4.23$, $p=0.04$]. We therefore examined whether the previous significant two-way interactions (gains x treatment & losses x treatment) were moderated by the probability of winning. There were no significant interactions between probability of winning and treatment when gains were large ($p=0.69$), gains were small ($p=0.94$), losses were large ($p=0.88$), and losses were small ($p=0.78$).

Figure 4. (A) Proportionate choice of the experimental gamble over the control gamble as a function of probability of winning (high, low), expected gains (large, small) and expected losses (large, small). **(B)** Mean deliberation times collapsed across all conditions.

(A)



(B)



Deliberation times

A significant interaction between probability of winning and gains [F(1,68)=23.5, p<0.001] was found; participants made faster choices when probability of winning was high and gains large and when probability of winning was low and gains small. The interaction between probability of winning and losses was also significant [F(1,68)=43.2, *p*<0.001]; participants made faster choices when probability of winning was high and losses were small, and when probability of winning was low and losses were large. The

three-way gains x losses x treatment interaction was marginally significant [F(1,68)=3.95, p=0.05]. Furthermore, across all conditions, the omega-3 group made choices significantly faster than the placebo group [F(1,68)=4.67, p=0.03] (figure 4b).

"Gains only" and "Losses only" Trials: Risk-seeking behaviour and deliberation times

Participants chose the guaranteed gain in gains-only trials significantly more often than the guaranteed loss in losses-only trials, thereby taking fewer risks when it comes to winning and risking when it comes to loosing [F(1,68) = 208.3; p < 0.001]. Treatment did not modulate this effect [F(1,68)=0.06 p=0.82]. Participants made significantly faster choices on the "gains-only" trials in comparison to the "losses-only" trials [F(1,68) = 78.4, p < 0.001]. Across trials, the omega-3 group made again their choices faster than the placebo group, but not significantly so [F (1,68) = 3.99; p > 0.05]. Table 4 displays the mean proportion of choices of the guaranteed outcome and the deliberation times for this choice for the two groups.

Table 4. Mean proportionate choice of the guaranteed outcome (in gains-only and losses-only trials) and mean deliberation times (± standard deviations (SD)) for the treatment and control groups.

	Mean Proportionate Choice ±SD		Mean deliberation times ±SD	
	Omega-3	Placebo	Omega-3	Placebo
Gains-only Trials	0.86 ±0.24	0.84 ±0.25	1786.9 ±928	2302.9 ±1302
Losses-only Trials	0.22 ±0.24	0.22 ±0.24	3003.2 ±1781	3913.5 ±2247

Discussion

The main findings of the present study were a positive effect of omega-3 supplementation on self-reported states of depression and tension and an effect on emotional decision-making. Effects of supplementation on the facial expression recognition test are difficult to interpret due to a parallel learning effect in both groups. In the affective attention test we found effects of supplementation in the omega-3 group but also in the placebo group. No significant effects were observed on memory, neutral attention, cognitive reactivity and depressive symptomatology.

We observed that supplementation with omega-3 PUFAs lead to a decrease of negative mood states such as tension and depression (POMS). A marginal effect of supplementation was also found on fatigue. However, the significant interactions were partly driven by the increase of these states in the placebo group. Prior studies in healthy individuals have found similar effects of omega-3 (high in EPA) supplementation on fatigue (Antypa et al., 2009) or on all mood states (Fontani et al., 2005) as measured with the POMS. In substance abusers, a decrease on tension scores using the POMS was also found after omega-3 (high in EPA) supplementation (Buydens-Branchey & Branchey, 2006). Su (2009) proposes a biological mechanism via which EPA may exert its effects on somatic symptoms of depression, like fatigue and tension. Animal studies show that EPA is important in balancing the immune/inflammatory functions by antagonizing membrane arachidonic acid (AA) and thus reducing prostaglandin E2 (PGE2) synthesis both of which are related to higher anxiety behaviour in rats (Song et al., 2003). EPA can suppress pro-inflammatory effects of AA and attenuate activation of PGE2 and thereby reduce stress and anxiety-like behaviour in rats (Song et al., 2004). Similarly, in humans, EPA may influence somatic manifestations of depression via its anti-inflammatory properties.

Although we found decreased scores of depression and tension as mood states in the omega-3 group, we did not observe a similar decrease in depressive symptoms (as measured with the BDI-II). One potential explanation for this is that the BDI-II is a clinical scale, which measures depressive symptoms and their severity and is less sensitive to change, especially in the lower spectrum of negative mood. The POMS is a measure of

general psychological distress and contains items that cover a wide range of negative mood states (eg. nervous, hopeless).

Furthermore, we observed that omega-3 supplementation modulated motivated decision-making. Overall, the omega-3 group made their choices significantly faster than the placebo group. The omega-3 group chose the experimental gamble less often when the expected gains were large and the expected losses were small, and this pattern of choice was unrelated to the perceived probability of winning (high or low). In previous studies this pattern of decision-making has been interpreted as signaling an impairment in the ability to discriminate between differences in magnitude of reward cues (Rogers et al., 2003) or punishment cues (Scarna et al., 2005). Those studies included healthy volunteers and studied the effects of tryptophan depletion (TD) (Rogers et al., 2003) and dopamine manipulation (Scarna et al., 2005) on risky decision-making. However, results on the effects of TD on decision-making are not unanimous. A study in healthy volunteers showed that tryptophan depleted participants chose the more likely of two possible outcomes significantly *more often* than controls thereby showing improved decision-making behaviour (Talbot et al., 2006). This was in contrast to previous findings that tryptophan depleted participants chose the more likely outcome significantly *less often* (Rogers, et al., 1999) or showed an imparment in discriminating between magnitude of rewards associated with different choices (Rogers, et al., 2003), thereby showing impaired decision-making in both studies.

Theoretically, after omega-3 supplementation one would expect an improvement in decision-making manifested as an increase in discrimination of the conditions in gambling outcomes (choice of gambles with high expected value). There are several speculative explanations for not observing such effects. Firstly, this is the first study to examine motivated decision-making in a group of recovered depressed individuals and consequently we have no prior data on baseline performance of this group to which we can make comparisons. Secondly, unmeasured intrinsic trait characteristics of the individuals may possibly explain the results. We have no pre-treatment measurement of this task and therefore we do not know whether groups differed at baseline and if effects can be confidently attributed to treatment. Most importantly, effects of genetic variability may also have affected performance. There is evidence that decision-making behaviour (using the same task) can be modulated by serotonergic polymorphisms (Roiser, Rogers, Cook, & Sahakian, 2006).

We found that the omega-3 group chose the fifty-fifty chance gamble more often than the placebo group in specific trials, thus showing a more "conservative" decision-making behaviour and took such decisions faster than the placebo group. In our previous study, we found that omega-3 supplementation promoted risk-seeking behaviour in the non-normative trials of the decision-making task compared to placebo in healthy volunteers (Antypa et al., 2009). In the present study, we found no effects of supplementation in the non-normative trials in recovered depressed individuals. Considering the residual neurobiological abnormalities that characterize recovered depressed patients often manifested as negative biases in information processing and emotional appraisal (Bhagwagar & Cowen, 2008), we did not expect comparable effects of omega-3 supplementation between the two studies. The higher cognitive reactivity levels in this sample, compared to those in healthy controls (Antypa et al., 2009), also indicate an increased cognitive vulnerability to depression.

Supplementation had minor effects on the other measures of emotional information processing. We found that the omega-3 group had a lower rate of omissions in the affective attention task and a lower reaction time during the shift blocks of the same task. However, the placebo group had a lower false alarm rate after supplementation in negative shift blocks. Although these differences yielded small to moderate effect sizes, these results cannot be reliably interpreted. Differences between the two groups were not statistically significant at post-test and supplementation had different effects on both groups in this task, which makes any interpretation dubious.

In the facial emotion recognition task, participants in the omega-3 group showed decreased recognition of the fearful emotion compared to the placebo group. This effect occurred particularly at the 50-60% intensity level of the fearful emotion. However, the interpretation of this effect is difficult because of a learning effect (figure 3). Subsequently, post-treatment differences between the two groups did not reach statistical significance. The observed effect is in line with previous literature showing that an increased recognition of fearful faces in recovered depressed patients in comparison to controls can be normalized after a single dose of citalopram (Bhagwagar et al., 2004). Our study provides only tentative evidence that omega-3 supplementation may affect facial emotion processing by decreasing fear recognition, but this finding is worthy of future investigation.

According to the neuropsychological hypothesis of anti-depressant drug action, anti-depressants modulate emotional information processing in a positive manner which

can lead to gradual changes in social reinforcement, behaviour and mood over time (Harmer et al., 2009). Evidence points toward a preceding effect of anti-depressant action on emotional information processing and a subsequent effect on mood, and changes in processing are associated with neural modulation in limbic areas and prefrontal circuitry (Harmer et al., 2009). Mechanistic explanations of potential anti-depressant action of omega-3 fatty acids mainly rely on neurochemical theories, largely based on animal models (eg. anti-inflammatory effects, brain-derived neurotrophic factor and neurotransmitter modulation, (Su, 2009; Owen et al., 2008; for reviews). We still lack information on the pathways that transpose neural changes to clinically meaningful effects, especially with regard to EPA mechanistic action. Recently, McNamara et al., (2010) provided the first evidence that DHA modulates functional activity in cortical attention networks in humans. Participants who had received 8 weeks of DHA supplementation showed significantly increased functional activation of the prefrontal cortex compared to those who had received placebo, during performance of a sustained attention task (McNamara et al., 2010). A plausible mechanism for DHA action in affective disorders is that DHA deficiency increases vulnerability to neuronal atrophy in the prefrontal cortex thus affecting multiple limbic structures (McNamara, 2010). For EPA, mechanisms at the neuro-cognitive level remain to be determined.

Although a few studies have found some effects of omega-3 supplementation on cognition (Antypa et al., 2009; Fontani et al., 2005; current study) it is far from clear whether the pathway to change in mood is via change in (emotional) cognition, as it is hypothesized with anti-depressant medication. The present study provided some evidence of omega-3 effects on mood and cognition however, more studies with thorough/rigorous designs are needed to answer the above question of directionality and shed light into pathways of action. Careful selection of outcome measures seems to play an important role. It is noteworthy that in the present study (as well as in Antypa et al., 2009) omega-3 supplementation had an influence only on the emotional information processing tasks of the test battery and not on the neutral ones. Furthermore, frequency of task administration may also affect results in pre-post designs. The decision-making task was administered only at post-test because order effects have been found (Wood et al., 2006). As evident from the present study, learning effects in the facial emotion recognition task may also interfere with observed results. Longer duration of supplementation may also be beneficial (Su et al, 2003; Peet & Horrobin 2002). More importantly, by investigating effects of supplementation in participants with abnormally

low EPA and DHA levels on their mood and cognition, we could further elucidate the mechanisms that bridge omega-3 PUFAs with depression.

Our study had a very low dropout rate and our omega-3 dosage was well tolerated and its consistency (high in EPA) is in line with previous positive trials using a minimum of 1g/day of EPA (Nemets et al., 2002; Peet & Horrobin, 2002; Su et al., 2003). However, some limitations need to be acknowledged. Firstly, results must be treated with considerable caution as the number of analyses for different outcomes can correspond to a high probability of Type I error (observing a chance finding). However, cognitive tasks measured different aspects of information processing with minor overlap. On the other hand, our sample size was large enough to detect moderate to large effects (d=0.65) with the conventional level of power (0.80) with an α=0.05.

This is the first study to examine effects of omega-3 supplementation in a group of recovered depressed patients, thus the present results may be considered as preliminary until replicated. Large, well-conducted studies are still necessary to further elucidate mood and cognitive effects of omega-3 fatty acids in participants with affective disorders and shed light into plausible neuropsychological mechanisms of action.

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