Omega-3 fatty acids and stress-induced changes to mood and cognition in healthy individuals

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A B S T R A C T

Omega-3 fatty acid (n-3 PUFA) intake is associated with improved mood and cognition, but randomized controlled trials addressing the causal nature of such relationships are less clear, especially in healthy, young adults. Stress is one potential mechanism by which n-3 PUFA may influence mood. Thus the present aim is to evaluate the influence of n-3 PUFA supplementation on stress-induced changes to mood, cognition, and physiological stress markers in healthy, young adults. Using a double-blind, placebo-controlled design, 72 young adults were randomized to receive 2800 mg/day fish oil (n = 36, 23 females) or olive oil control (n = 36, 22 females) for 35 days. Subjects completed measures of mood and cognition before supplementation, and two times after supplementation: following an acute stressor or non-stressful control task. The stress induction was effective in that the stressor impaired mood, including augmenting feelings of tension, anger, confusion and anxiety, reduced accuracy on a cognitive task measuring attentional control and the ability to regulate emotion, and increased salivary cortisol and pro-inflammatory cytokine interleukin-1β (IL-1β). Rated anger and confusion increased with stress in the olive oil group, but remained stable in the fish oil group. However, fish oil had no further effects on mood, cognitive function, cortisol, or IL-1β. Fish oil exerted few effects in stressful and non-stressful situations, consistent with findings showing little influence of n-3 PUFA supplementation on mood and cognition in young, healthy individuals. Potential target populations who would more likely benefit from increased n-3 PUFA intake are discussed.

1. Introduction

Polyunsaturated fatty acid (PUFA) levels play a role in neurological and psychological functions (Dacks et al., 2013; Parker et al., 2006). Omega-3 (n-3) and omega-6 (n-6) fatty acids are the main constituents of the PUFA family. n-6 PUFAs include linoleic acid (LA) and arachidonic acid (ARA) and n-3 PUFAs include alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Parker et al., 2006).

1.1. n-3 PUFAs and mood and cognition

Extant evidence investigating the potential antidepressant effects of n-3 PUFA supplementation is generally split between studies finding a beneficial influence in individuals with major depressive disorder and those reporting null results (for reviews, see Parker et al., 2006; Martins, 2009; Lin et al., 2010; Giles et al., 2013). Only a few published empirical studies have assessed the effect of n-3 PUFA supplementation on mood in young, healthy populations. Fontani et al. (2005a,b) assessed the influence of 2.8 g/day n-3 PUFAs (2:1 EPA:DHA) on mood using the Profile of Mood States (POMS). They found increased feelings of vigor and reduced feelings of anger, anxiety, fear, depression, and confusion in the n-3 PUFA group compared to placebo (Fontani et al., 2005a,b). However, a more recent study found that 2.3 g/day n-3 PUFA (approximately 7:1 EPA:DHA) reduced feelings of POMS fatigue only (Antypa et al., 2009).

The majority of research into cognitive effects of n-3 PUFA supplementation has occurred at either end of the lifespan: in infants and older adults (Giles et al., 2014b). However, less work has examined n-3 PUFAs and cognition in the middle of the lifespan, when dietary supplement use may be highest (e.g., Briefel and Johnson, 2004), and the results are equivocal. In young adults, some studies show that n-3 PUFA supplementation enhanced verbal learning (Karr et al., 2012) and episodic and working memory (Stonehouse et al., 2013). However, another study suggests that n-3 PUFA supplementation did not influence cognitive performance across a range of tasks measuring response inhibition, facial expression recognition, and immediate and delayed recall (Antypa et al., 2009). Further, n-3 PUFA supplementation improved response inhibition and sustained attention in one experiment (Fontani et al., 2005b), but not others (Antypa et al., 2009; Hamazaki et al., 1996, 1999; Karr et al., 2012; Stonehouse et al., 2013).

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Perhaps contributing to the mood and cognitive effects described above, additional evidence suggests that n-3 PUFA intake may influence neural function. In one study of healthy 8–10 year old boys, DHA supplementation increased functional activation in the dorsolateral prefrontal cortex (DLPFC) during a sustained attention task (McNamara et al., 2010). In addition, erythrocyte DHA composition was positively correlated with DLPFC activation. The DLPFC plays a role in working memory (Cohen et al., 1997; Miller et al., 1996) and task switching (Dove et al., 2000; Konishi et al., 2003) and previous neuroimaging studies indicate hypo-activation in the DLPFC in individuals with depression compared to healthy controls (for review, see Koenigs and Grafman, 2009). Thus n-3 PUFA supplementation may influence DLPFC-associated cognitive function, including mood and emotion-related cognitive processing.

1.2. n-3 PUFAs and stress

Mixed findings for mood and cognitive effects of n-3 PUFA supplementation may be due, in part, to variations in the degree to which subjects experienced stress. While some studies evaluated post-supplementation measures during periods of stress (Hamazaki et al., 1996, 1999), others did not manipulate stress (Antypa et al., 2009; Fontani et al., 2005a,b). Stress may be one pathway by which n-3 PUFA levels modulate mood and cognition. Stress and depression, as well as dietary composition akin to the Western diet with high n-6 to n-3 PUFA ratio, have been shown to influence inflammation through the same pathways (Kiecolt-Glaser, 2010). For instance, stress and depression increase pro-inflammatory cytokine production, a broad class of polypeptide mediators that are secreted by cells of the body, often as part of an immune response, including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF) (O’Brien et al., 2004). In healthy young adults, three weeks of n-3 PUFA supplementation eliminated stress-induced cortisol increase and dampened epinephrine increase in response to an experimentally-induced stressor (Delarue et al., 2003). To our knowledge, this is the only study to measure the influence of n-3 PUFA on physiological responses to stress, and suggests that n-3 PUFA supplementation may influence stress-induced changes to mood in healthy individuals.

Animal studies suggest that n-3 fatty acids mitigate stress-induced cognitive impairments. n-3 PUFA supplementation in γ-irradiation- and cerebral ischemia-damaged rats reduced levels of reactive oxidative species and number of apoptotic neurons in the hippocampus (Su, 2010). Further, an n-3 deficient diet is associated with learning deficits and heightened anxiety (Heinrichs, 2010) and n-3 PUFA supplementation in rats prevented anxiety- and depressive-like behaviors and learning and memory deficits induced by stress (Ferraz et al., 2011). Similarly, cod liver oil-containing DHA and EPA reduced rats’ restraint stress-induced impairments on recall and spatial memory (Trofimiuk and Braszko, 2011). However, it should be noted that the relative dosage in animal studies which is generally higher than that in humans, e.g., 525–900 mg/kg/day EPA + DHA in rats (Ferraz et al., 2011; Trofimiuk and Braszko, 2011) would equal approximately 35.7–61.2 g/day EPA + DHA in a 68 kg human, well above the recommended 3.5 g/day EPA + DHA (Kris-Etherton et al., 2003).

1.3. The present study

Preliminary evidence exists for the anti-depressive actions of n-3 PUFA (Giles et al., 2013; Martins, 2009; Parker et al., 2006) and anti-stress actions in humans (Delarue et al., 2003) and rodents (Ferraz et al., 2011). However, to date, there have been no published empirical studies examining the effects of n-3 PUFA supplementation on stress-induced changes in mood and cognitive behavior in young, healthy individuals. Given that the Western diet has shifted towards greater intake of n-6 PUFAs at the expense of intake of n-3 PUFAs (Kiecolt-Glaser, 2010; Parker et al., 2006), these questions warrant further empirical study.

The primary objective was to evaluate the influence of n-3 PUFA supplementation on stress-induced changes in mood. Based on available evidence that n-3 PUFA supplementation improves mood (Antypa et al., 2009; Fontani et al., 2005a,b) and ameliorates physiological stress responses (Delarue et al., 2003), we expected that n-3 PUFA supplementation would result in enhanced mood relative to control supplementation, and that stress would impair mood but that n-3 PUFA would reduce this impact relative to control supplementation.

Preliminary evidence suggests that n-3 PUFA supplementation influences cognitive performance (Fontani et al., 2005b; Karr et al., 2012; Stonehouse et al., 2013) and associated brain regions, i.e., the DLPFC (McNamara et al., 2010), therefore a second objective was to determine the impact of n-3 PUFA supplementation on emotion-associated cognitive processing. We chose two tasks to evaluate a range of such processing, including the Emotional Interference Task (EIT; Dolcos and McCarthy, 2006) and Morphed Faces Task (MFT; Brunyé et al., 2013; Joormann and Gotlib, 2006; Young et al., 1997), which have been shown to activate brain regions similar to those influenced by n-3 PUFA supplementation, including the DLPFC (Dolcos and McCarthy, 2006). Given that DHA supplementation enhanced prefrontal activation associated with cognitive processing, we hypothesized that n-3 PUFA supplementation would enhance performance on these tasks.

The present study also sought to quantify the interactive effects of n-3 PUFA and stress on salivary cortisol, a biomarker of arousal (Hellhammer and Schubert, 2012) and the pro-inflammatory cytokine interleukin 1β (IL-1β), a biomarker of inflammation (Calder, 2010). We expected that stress would increase both salivary cortisol and IL-1β, but that this effect would be less pronounced following n-3 PUFA than control supplementation.

2. Methods

Seventy two individuals participated for monetary compensation. All the participants were in good health, and did not use nutritional supplements or prescription medication other than oral contraceptives. Written informed consent was obtained, and all procedures were approved by the Tufts University Institutional Review Board. This study used a double-blind, mixed-factor, repeated measures design approved by the Tufts University Institutional Review Board. Seventy two individuals participated for monetary compensation. All the participants were in good health, and did not use nutritional supplements or prescription medication other than oral contraceptives. Written informed consent was obtained, and all procedures were approved by the Tufts University Institutional Review Board. This study used a double-blind, mixed-factor, repeated measures design approved by the Tufts University Institutional Review Board.

2.1. Omega-3 administration

In order to control for taste, FO and OO were administered in capsule form (developed by Dr. Michael Roberge, RPh, Compounded Solutions, Monroe, CT). Capsules contained a total of either 2800 mg FO (1680 mg EPA, 1120 mg DHA) or 2800 mg OO (control), divided into 7 capsules per day. All capsules appeared identical in shape and size. At the end of the final test session, subjects were asked which treatment they thought they had received (answer choices: “Fish Oil,” “Olive Oil,” or “Not sure”). They were then asked if they experienced any side effects from their treatment (answer choices: “Yes” or “No”), and if “Yes,” to list these side effects.

2.2. Diet record

Participants reported their dietary intake of foods that contain n-3 and n-6 PUFAs once per week throughout the duration of the study (Tufts University School of Medicine, Nutrition Infection Unit, 2002). Foods included fish (e.g., salmon, sardines, tuna), nuts and seeds (e.g., almonds, walnuts, flax seed), oils (e.g., walnut oil, soybean oil, flax seed oil), grains and beans (e.g., soybean oil, tofu) and greens (e.g., spinach, kale, collard greens). One serving size equaled 4 oz (fish and tofu), 1 oz (nuts), 1 tablespoon (oils), and 1/2 cup (soybeans and greens). The subjects were asked to report the number of servings of each food that
they had eaten in the past week, and were given examples of serving sizes for reference (e.g., 4 oz equals a bar of soap; Sugar, 2007).

2.3. Mood measures

2.3.1. Profile of Mood States (POMS) Questionnaire

The POMS is an inventory of subjective mood and arousal states (McNair et al., 1971). Participants rate a series of 65 mood-related adjectives on a five point scale, which factor into six subscales: tension, depression, anger, vigor, fatigue, and confusion as well as a composite score of total mood disturbance (Lieberman et al., 1996).

2.3.2. State–Trait Inventory for Cognitive and Somatic Anxiety (STICSA)

The STICSA consists of independent State and Trait scales, each composed of 21 self-report items (Ree et al., 2008). The scale asks how participants feel in response to a number of somatic (e.g., dizziness, fast breathing, clammy palms) and cognitive anxiety symptoms (e.g., lack of concentration, worry, trouble remembering).

2.4. Salivary cortisol and interleukin-1β

Saliva was collected for analyses of salivary cortisol and the pro-inflammatory cytokine interleukin-1β (IL-1β) through passive drool. Samples were analyzed in duplicate in an independent laboratory (Salimetrics LLC, State College, Pennsylvania).

2.5. Stress manipulation: Trier Social Stress Test (TSST)

The TSST is a 20-minute psychosocial stress task consisting of 3 stages: (1) 10-minute preparatory stage, (2) 5-minute public speaking task, and (3) 5-minute mental arithmetic task (Kirschbaum et al., 1993). It is a commonly used method of effectively, experimentally inducing psychosocial stress (Giles et al., 2014a). The control condition consisted of a 5 minute speech (about a movie or a book) and 5 min of mental arithmetic, both completed in an empty room. This control condition is relatively similar in physical and mental workload but lacks the stress-inducing components of the TSST (i.e., social evaluative threat and uncontrollability; Kuhlmann et al., 2005). To ensure the efficacy of the stress manipulation, subjects were blinded to its true purpose, i.e., to induce stress, and were told that the task was intended to assess verbal communication ability. Participants were fully debriefed as to the true intention of the TSST upon completion of the study.

2.6. Cognitive tasks

2.6.1. Emotional Interference Task (EIT)

The EIT task is a modified Sternberg item recognition paradigm using visuospatial stimuli of abstract shapes (Erk et al., 2007; Wolf and Walter, 2005), which measures attentional control and the ability to regulate emotion. Participants complete a delayed match-to-sample task, with distracters presented during the delay period (Dolcos and McCarthy, 2006). During each trial, three visuospatial stimuli appear during the initial stimulus presentation, and then two scrambled, neutral or negative distracter images are presented immediately prior to a recognition task. Dependent measures include accuracy and response time in identifying whether the target visuospatial stimuli presented after the distracter matches one of the three initial visuospatial stimuli.

2.6.2. Morphed Faces Task (MFT)

The MFT involves the dynamic onset and offset of six depicted facial emotions: anger, disgust, happiness, fear, sadness, and surprise (i.e., Brunyé et al., 2013; Joormann and Gotlib, 2006; Young et al., 1997). During each trial, 100-frame animations (at approximately 50 frames per millisecond) of faces either depicted the gradual onset (from neutral) or the gradual offset (to neutral) of an emotional expression. Participants pressed the spacebar when they believed that the face displayed the target emotion (during onset trials) or a neutral expression (during offset trials). Upon spacebar press, the face paused on the current frame, and participants rated the emotional intensity of the paused face on a scale from 1–7. Half of the trials depicted emotional expression onset, and half offset. Dependent measures included (1) a stop frame at which the participant considered the face to express the target emotion and (2) rating of emotional intensity of stopped face. Onset and offset trials were equated by subtracting offset stop frames from 100, such that lower stop frames signify higher sensitivity to the target emotion.

2.7. Procedure

Participants completed four sessions on separate days: one practice session 2–7 days before beginning supplementation to become familiarized with the experimental procedure and tasks, and three test sessions, one before supplementation (Day 0) and two after supplementation (Days 34 and 35). The practice and test sessions took place in the morning; beginning between 0700–0900 h. Start times were consistent within each participant.

During test sessions, participants completed baseline measures of the POMS and STICSA-S and provided a saliva sample for salivary cortisol and IL-1β analysis. During the first test session, the participants then completed the EIT and MFT, followed by a second set of questionnaires and provision of a saliva sample. After the first test session, participants consumed FO or OO every day for 35 days. To promote treatment adherence, participants came to the lab each weekday and took the capsules in front of the experimenter. On weekends, participants took the capsules on their own, and were required to send a text message to the experimenter when they did so. Of the 72 subjects, five subjects in the FO group and six subjects in the OO group missed one day of supplementation over the course of the five-week study, and one subject in the OO group missed three days, resulting in compliance rates of 99.6% in the FO group and 99.3% in the OO group.

The final two test sessions took place on the 34th and 35th days of supplementation and were identical to the first session with the addition of the stressful or non-stressful TSST, in counterbalanced order, and a third saliva sample 60 min after the TSST (see Fig. 1 for a schematics of the procedure). Participants also completed a Cognitive Reappraisal Task (Urry, 2009) after the EIT and MFT on days 34 and 35, but data will not be reported due to technical problems with the task. The Cognitive Reappraisal Task involves viewing a series of neutral and unpleasant images while attempting to either reappraise or maintain their thoughts of the images.

![Fig. 1. Schematic representation of the schedule for each study session. During the study sessions, participants first completed baseline measures of the Profile of Mood States (POMS) and State–Trait Inventory for Cognitive and Somatic Anxiety (STICSA-S) and provided saliva samples for analysis of cortisol and the pro-inflammatory cytokines interleukin-1β (IL-1β). They then completed the stressful or non-stressful Trier Social Stress Test (TSST), followed by the POMS, STICSA-S, and saliva (post-supplementation on days 34 and 35 only). They then completed the Emotional Interference Task (EIT) and Morphed Faces Task (MFT) and the final saliva sample.](image-url)
2.8. Statistical methods

First, to determine whether the FO and OO groups differed on any measures prior to supplementation, all pre-supplementation measures were subjected to univariate analysis of variance (ANOVA)s with supplementation 2(FO, OO) as the between-subjects factor and alpha levels adjusted using the Bonferroni correction. The two POMS and STICSA time-points pre-supplementation were averaged and then compared.

Change scores were then calculated for each POMS and STICSA sub-scale on both the stressful and non-stressful TSST sessions (post-TSST = pre-TSST), such that positive values indicate an increase from pre-TSST and negative values indicate a decrease from pre-TSST. Calculated in this way, change scores account for pre-treatment differences in the STICSA-S. The POMS and STICSA as well as IL-1β, EFT and MFT were then analyzed to determine the difference between the stressful and control post-supplementation sessions using repeated measures ANOVAs with group 2(FO, OO) as the between subjects factor and stress 2(Stress, Control) as the within subjects factor.

Cortisol was analyzed in a similar manner, with the additional between-subjects factor of time 3(pre-TSST, 10 min post-TSST, 60 min post-TSST). Because similar studies found that salivary cortisol concentrations had skewed distributions (Schoofs et al., 2008), we tested for normality using the Lilliefors procedure. Cortisol and IL-1β data showed a positively skewed distribution, and therefore were log-transformed. The ANOVAs were performed with the transformed data.

Given that previous studies found inverse relationships between fish intake and depression in women only (Colangelo et al., 2009; Sanchez-Villegas et al., 2007; Tanskanen et al., 2001; Timonen et al., 2004), exploratory analyses also included gender 2(Male, Female) as a between subjects factor. Unless otherwise noted (see the Demographic Information section), exploratory analyses also included gender 2(Male, Female) as a between subjects factor. Factorial ANOVAs with group 2(FO, OO) as the between subjects factor and alpha levels adjusted using the Bonferroni correction were conducted. All statistical analyses were performed using SPSS 17.0.

3. Results

3.1. Preliminary analyses

3.1.1. Demographic Information

Table 1 shows demographic information, dietary n-3 and n-6 PUFA intake, and trait cognitive and somatic anxiety for the FO and OO groups. Groups did not differ on any of the measures (all ps > .14).

3.1.2. Blinding and side effects

Of the 36 subjects in each group, 30 (83%) subjects in the FO group correctly guessed their treatment assignment. Subjects given FO were able to guess their treatment group above chance. Side effects included fishy burps and aftertaste (n = 6 FO, n = 0 OO), queasiness, upset stomach and bloating (n = 1 FO, n = 4 OO), loose or discolored stools (n = 0 FO, n = 2 OO) and headaches (n = 1 FO, n = 1 OO).

3.1.3. Baseline group differences

Table 2 shows baseline between group differences for all mood, cognitive and physiological measures. No single comparison reached full or marginal significance with one exception: STICSA state cognitive anxiety was higher in the FO than that in the OO group.

3.1.4. Stress effects on mood

POMS data reflects 71 subjects (n = 36 FO, 35 OO) as one subject failed to complete instrumentation following the stressful TSST (Table 3). Stress increased POMS subscales for tension (F(1,70) = 33.468, p < .001 (η² = .339), depression (F(1,70) = 6.838, p < .05 (η² = .094), anger (F(1,70) = 10.865, p < .01 (η² = .095), confusion (F(1,70) = 17.817, p < .001 (η² = .150), and total mood disturbance. More specifically, rated tension increased pre- to post-stressful TSST t(71) = 5.355, p < .001 (d = .631) but decreased pre- to post-non-stressful TSST t(71) = −2.308, p < .05 (d = .272) (Stress = 3.65 ± .68, Non-Stress = −1.03 ± .44). Rated depression did not change pre- to post-stressful TSST (p > .51) but decreased pre- to post-non-stressful TSST t(71) = −2.794, p < .01 (d = .329) (Stress = .41 ± .59, Control = −1.28 ± .46). Rated anger increased pre- to post-stressful TSST t(71) = 2.644, p < .05 (d = .312) but decreased pre- to post-non-stressful TSST t(71) = −2.144, p < .05 (d = .253) (Stress =

Table 1

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<thead>
<tr>
<th></th>
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<th>OO Mean ± SD</th>
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No differences in age, body mass index (BMI), habitual exercise, weekly n-3 or n-6 PUFA intake or trait anxiety between the fish oil (FO) and olive oil (OO) groups pre-supplementation.
2.04 ± 0.75, Control = −.89 ± .42). Rated confusion increased pre- to post-stressful TSST (t(71) = 4.088, p < .001 (d = .482) but did not change pre- to post-non-stressful TSST (p > .07) (Stress = 1.98 ± .48, Control = −.59 ± .31). The total mood disturbance composite score increased pre- to post-stressful TSST (t(71) = 2.829, p < .01 (d = .333) but decreased pre- to post-non-stressful TSST (t(71) = −3.005, p < .001 (d = .354) (Stress = 6.87 ± 2.39, Non-stress = −5.06 ± 1.70). No main effects or interactions were found for rated vigor or fatigue (all ps > .38).

Stress also increased STICSA somatic F(1,70) = 14.989, p < .001 (η² = .108) and cognitive anxiety F(1,70) = 4.924, p < .05 (η² = .045). Somatic anxiety increased pre- to post-stressful TSST (t(71) = 2.299, p < .05 (d = .271) and decreased pre- to post-non-stressful TSST (t(71) = −2.712, p < .01 (d = .320) (Stress = 0.67 ± 0.29, Non-stress = −0.78 ± 0.27; Table 4). Cognitive anxiety did not change pre- to post-stressful TSST (p > .35) but decreased pre- to post-non-stressful TSST (t(71) = −3.041, p < .01 (d = .358) (Stress = 0.46 ± 0.49, Non-stress = −0.78 ± 0.26).

3.1.5. Stress effects on cognitive performance

Accuracy on the EIT was lower following the stressful than non-stressful TSST F(1,70) = 5.117, p < .05 (η² = .013) (Stress = 0.88 ± 0.02, Control = 0.91 ± 0.01). Stress did not influence response time (p > .2).

Analysis of rated intensity on the MFT revealed an Emotion by Stress by Gender interaction F(5,330) = 3.838, p < .01 (η² = .002). For happy faces F(1,160) = 14.253, p < .001 (η² = .081) females rated happy faces as more intense following the stressful TSST (p < .01) whereas males rated happy faces as more stressful during the non-stressful TSST (p < .05). No differences were found for other emotional expressions.

Table 3
Profile of Mood States (POMS) change score (post−pre−Trier Social Stress Test) means (SEM) for each treatment combination (n = 71; 36 FO, 35 OO).

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Stress increased rated tension (p < .001), anger (p < .01), confusion (p < .001), and total mood disturbance (p < .001). Rated anger remained stable in the fish oil (FO) and increased in the olive oil (OO) group (p < .05) and rated confusion remained stable in the FO group across both stressful and non-stressful TSST but increased in the OO group during the stressful TSST (p < .05; see Fig. 2). No other main effects or interactions were found between FO and OO group, and no effects were found for rated vigor or fatigue (all ps > .38).
somatic anxiety increased during the stressful and decreased during the non-stressful test session.

Stress increased rated state somatic (p < .001) and cognitive (p < .05) anxiety. Rated state somatic anxiety increased during the stressful and decreased during the non-stressful TSST in the fish oil (FO) but not in the olive oil (OO) group (p < .01; see Fig. 3). No other main effects or interactions were found (all ps > .11).

(All ps > .12). For stop frames, i.e., the number of frames passed before pressing the space bar, an Emotion by Gender interaction \(F(5,340) = 6.099, p < .001 (\eta^2 = .012)\) showed lower stop frames, i.e., higher sensitivity to emotional expression, to fearful (p < .05) and angry (p < .01) faces in females than males, and higher stop frames to surprised (p < .01) and disgusted (p < .05) faces in females than males. No differences were found for happy (p > .08) or sad (p > .05) faces. No main effects of Stress or Stress by Emotion interactions were found (all ps > .30).

### 3.1.6. Stress effects on cortisol and IL-1β

Cortisol data reflects 71 subjects (n = 36 FO, 35 OO) as one subject failed to provide sufficient saliva (Table 5). Cortisol was higher during the stressful than non-stressful test session \(F(1,69) = 9.803, p < .01 (\eta^2 = .012)\) (Stress = .38 ± .03, Non-stress = .32 ± .02). Further, cortisol was higher 10-min after the TSST than before or 60-min after the test session \(F(1,213) = 33.653, p < .001 (\eta^2 = .154)\) (pre-TSST = .37 ± .03, 10 min post-TSST = .40 ± .02, 60 min post-TSST = .26 ± .02). A main effect of Gender \(F(1,167) = 9.614, p < .01 (\eta^2 = .057)\) showed that cortisol levels were higher in females than those in males (Female = .40 ± .03, Male = .27 ± .06). No Time × Stress interactions were found for cortisol (p > .1).

IL-1β (in pg/mL) was higher during the stressful than the non-stressful test session \(F(1,70) = 4.140, p < .05 (\eta^2 = .011)\) (Stress = 190.96 ± 24.09, Non-stress = 227.27 ± .7422; Table 6).

### 3.2. Primary analyses

#### 3.2.1. Does n-3 PUFA supplementation improve mood and reduce the effect of stress on mood relative to control supplementation?

On the POMS, a main effect of diet Group was found for anger \(F(1,70) = 1.949, p < .05 (\eta^2 = .018)\), in which rated anger did not change pre- to post-TSST in the FO (p > .50) but increased pre- to post-TSST in the OO group \(t(36) = 2.243, p < .05 (d = .379)\) (FO = −34.57 ± 57, OO = 1.49 ± 59). A Stress × Group interaction for confusion \(F(1,70) = 4.345, p < .05 (\eta^2 = .067)\) showed that feelings of confusion increased pre- to post-stressful TSST \(t(34) = 3.866, p < .001 (d = .653)\) and decreased pre- to post-non-stressful TSST in the OO group \(t(34) = 4.088, p < .001 (d = .410)\) but did not change pre- to post-stressful or non-stressful TSST in the FO (p > .05) group (Fig. 2).

Although ANOVAs were performed on log-transformed data, the table shows raw data. Salivary IL-1β was higher during the stressful than the non-stressful test session (p < .05). No effects were found for Group (p > .06) or Stress × Group (p > .19).

3.2.2. Does n-3 PUFA supplementation improve cognition and reduce the effect of stress on cognition relative to control supplementation?

No effects for diet Group or Stress × Group were found on the EIT or MFT (all ps > .12).

3.2.3. Does n-3 PUFA supplementation reduce cortisol and IL-1β and reduce the effect of stress on salivary cortisol and IL-1β relative to control supplementation?

No effects for diet Group, Time × Group, Stress × Group or Stress × Time × Group were found for cortisol or IL-1β (all ps > .07).

### 4. Discussion

We evaluated the influence of n-3 PUFA supplementation on stress-induced changes to mood and emotion-related cognitive processing in healthy young adults. We found that the stress manipulation exerted effects consistent with the stress literature, including impaired mood and anxiety and increased cortisol and IL-1β. However we detected limited effects of n-3 PUFA supplementation on mood, cognition, or physiological responses either as a main effect or in interaction with stress.

4.1. n-3 PUFA influence on mood

Stress impaired mood, including augmenting feelings of tension, anger, confusion and cognitive and somatic anxiety. Rated anger and confusion increased with stress in the OO group, but remained relatively stable in the FO group. However, the opposite was true of somatic anxiety, as rated somatic anxiety increased with stress in the FO but...
mixed, with some studies finding no effects on mood and did not support our hypothesis that FO supplementation would improve mood, in stressful or non-stressful circumstances.

These results are generally in line with previous epidemiological studies and randomized controlled trials. For instance, prospective and cross-sectional studies assessing the association between n-3 PUFA intake and depressive mood within the general population are quite mixed, with some studies finding an inverse association between fish or n-3 PUFA intake and depressive symptoms (Appleton et al., 2007; Astorg et al., 2008; Panagiotakos et al., 2010), but others finding no relationship between n-3 PUFA intake and depressive symptoms (Hakkarainen et al., 2004; Murakami et al., 2008). Similar to results from epidemiological studies, those from randomized controlled trials assessing the influence of PUFA supplementation on depressive symptoms are divided between those reporting beneficial effects (da Silva et al., 2008; Lesperance et al., 2011; Lucas et al., 2009; Nemets et al., 2002; Peet and Horrobin, 2002; Su et al., 2003) and no influence (Carney et al., 2009; Grenyer et al., 2007; Marangell et al., 2003; Rogers et al., 2008; Silvers et al., 2005). Thus the present study supports a number of previous findings across epidemiological studies as well as randomized controlled trials showing limited effects of n-3 PUFA supplementation on mood in stressful or non-stressful situations.

4.2. n-3 PUFA influence on cognition

Likewise, n-3 PUFA supplementation had no effects on emotion-related cognitive processing. n-3 PUFA supplementation did not affect performance on the Emotional Interference Task or Morphed Faces Task. We predicted that FO would improve performance on these tasks relative to OO supplementation based on previous findings that n-3 supplementation benefited other cognitive tasks including response inhibition, sustained attention, and verbal learning (Fontani et al., 2005b; Karr et al., 2012). However other studies support our null results finding that n-3 PUFA supplementation had no influence on cognitive processes on response inhibition, facial expression recognition, and memory (Antypa et al., 2009; Karr et al., 2012). Unlike mood, inconsistent effects on cognition may be due to differences in dose as well as the EPA to DHA ratio in fish oil, as fish oil relatively high in DHA content, e.g., 1:5 EPA:DHA, enhanced performance on the Stroop task measuring selective attention as response inhibition (Jackson et al., 2012a) and oxygenation in the prefrontal cortex during the Stroop task (Jackson et al., 2012b). Our 1:5 EPA:DHA ratio was chosen based on previous reviews that suggested that EPA has greater antidepressant efficacy than DHA (Martins, 2009; Parker et al., 2006), but it may be the case that our DHA dose was insufficient to produce cognitive benefits.

4.3. n-3 influence on physiological stress response

Previous evidence that n-3 PUFA intake may influence mood via a mechanism involving inflammation (Sinclair et al., 2007) suggests that n-3 PUFA supplementation may mediate stress induced changes to mood, cognition, and physiological stress response. To this end, we found increased salivary cortisol and IL-1β (Kirschbaum et al., 1993; Steptoe et al., 2001) but contrary to extant evidence, n-3 supplementation did not influence stress-induced effects on cortisol or IL-1β (Calder, 2006; Delarue et al., 2003). As discussed in the following section, selection of acute rather than chronic stress as well as high baseline n-3 PUFA consumption may at least partially account for limited n-3 PUFA effects on HPA activation and inflammation.

4.4. Limitations

A primary limitation to the present study is the lack of erythrocyte fatty acid analysis. Erythrocyte PUFA levels are associated with, and may influence, mood changes including depressive symptoms. For instance, higher erythrocyte DHA + EPA and lower AA:EPA levels have been associated with improved depressive symptoms (Sinn et al., 2011). This is consistent with recent work from Meyer et al. (2013) who found that erythrocyte DHA and DHA + EPA, but not EPA alone, were associated with improved depressive symptoms, in the absence of a between-group effect of n-3 PUFA versus control supplementation (Meyer et al., 2013).

A second limitation is the lack of adequate blinding. 83% of the subjects in the FO and 44% of the subjects in the OO group correctly guessed their treatment assignment. Unlike in the present study in which no additional oils or flavors were added to either treatment, previous studies assessing omega-3 influences on mood, particularly depression, took measures to enhance treatment blinding such as adding lemon or orange flavor to both fish oil and placebo capsules (Antypa et al., 2009; Nemets et al., 2002; Rogers et al., 2008; Silvers et al., 2005) and adding trace amounts of fish oil to placebo capsules (Freeman et al., 2008; Lesperance et al., 2011; Lucas et al., 2009). Future trials should keep study blinding in mind, and either add additional oil or flavors to better equate the aftertaste or fishy burps.

Third, the sample size and supplementation duration may have curtailed the findings. The sample size and supplementation duration exceed the majority of similar studies assessing the influence of n-3 PUFA supplementation on mood and cognition in healthy individuals (Antypa et al., 2009; Fontani et al., 2005a,b; Karr et al., 2012). However the mean diet Group effect size was small (i.e., mean between-subjects $η^2 = .003$), indicating that the current sample size may have been too low to detect significant effects between the FO and OO groups. Elevations in EPA and DHA in red blood cells have been found after 2 and 4 weeks of fish oil supplementation, respectively (Barcelo-Coblijn et al., 2008), and red blood cell fatty acid levels may correlate to those
in the cerebral cortex (Carver et al., 2001). However, longer supplementation periods akin to those employed in numerous randomized controlled trials, e.g., 8–12 weeks (da Silva et al., 2008; Lucas et al., 2009; Su et al., 2003, 2008) are warranted to allow sufficient time for elevations in PUFA composition of the cerebral cortex.

Other limitations do not necessarily pertain to the study design but rather to how best to determine the extent to which n-3 PUFAs influence mood and cognition, particularly in times of stress. First, young, healthy students may not benefit from n-3 PUFA supplementation as much as other populations. Mean n-3 PUFA intake ranged from approximately 8–13 g per week among the foods evaluated in the present sample. The American Heart Association recommends eating two servings of fatty fish per week, in addition to ALA-rich foods such as flax seed, walnuts and canola oil. Although fish range in n-3 PUFA content according to type and whether they are farm-raised or wild-caught, on the upper end of the spectrum, two servings of pink salmon provide approximately 2.18 g/week EPA + DHA (Kris-Etherton et al., 2003), meaning that subjects in the present study consumed well over minimum n-3 PUFA intake requirements.

Second, n-3 PUFA supplementation may counteract impairments due to chronic stress, rather than acute stress as assessed in our study by using the Trier Social Stress Test. Despite research showing that acute stressors such as the Trier Social Stress Test indeed increase pro-inflammatory cytokines, individuals undergoing more chronic-type stressors which induce longer-lasting elevations in proinflammatory cytokines may be more apt to benefit from the anti-inflammatory actions of n-3 PUFA supplementation (Brydon et al., 2005; Steepe et al., 2001; Yamakawa et al., 2005).

Finally, OO is most often used as the control treatment in comparison to n-3 PUFA-rich FO in randomized controlled trials. OO is primarily comprised of monounsaturated fat (MUFA), which may also have beneficial changes in mental health. For instance, higher MUFA intake was associated with reduced cognitive decline in older adults (Naqvi et al., 2011; Okereke et al., 2012; Sofrizzoni et al., 2008; Veracambre et al., 2010) and reduced depressive symptoms in older adults (Kyrozis et al., 2009). Thus comparing FO to OO supplementation may mask some beneficial effects of FO on mood and cognition.

5. Conclusion

n-3 PUFA supplementation is thought to mediate stress induced changes to mood, cognition, and physiological stress response. However we found that fish oil supplementation did little to negate impairments in mood, emotion-related cognitive processing, and physiological stress markers, including cortisol and IL-1β. While some aspects of mood, including anger and confusion, tended to remain more stable in individuals supplemented with fish oil than those supplemented with olive oil, these moods effects were insufficient to conclude any anti-stress benefits of n-3 PUFA.

There is considerable debate in the literature as to whether n-3 PUFA intake confers any mood or cognitive benefit. A large body of research has explored the potential antidepressant effects of n-3 PUFA intake, and findings from epidemiological studies and randomized controlled trials are mixed (for a review, see Giles et al., 2013). To date most studies assessing the cognitive effects of n-3 PUFA supplementation have focused on infants, children and older adults, and only a handful of studies have evaluated the relationship in young adults. Despite lack of clear evidence that n-3 PUFA intake improves mood or cognitive processing, multiple methodological limitations in extant literature may mask beneficial effects. Additionally, young healthy individuals such as those in the present study may not be an ideal target population in addressing potential mood and cognitive benefits of n-3 PUFA intake. Individuals who are older, have a mood disorder such as major depressive disorder, or have initially low n-3 PUFA levels may comprise populations who could benefit from n-3 PUFA intake. Future research should address methodological limitations limiting extant research, and specify target populations for whom n-3 PUFA supplementation may be most helpful.

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References


