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

A thesis

submitted by

Grace E. Giles

In partial fulfillment of the requirements

for the degree of

Master of Science

in

Psychology

TUFTS UNIVERSITY

August, 2013

Adviser: Robin B. Kanarek, Ph.D.

ABSTRACT

Omega-3 fatty acid (*n*-3 PUFA) intake is associated with improved mood and cognition, especially in depressed and older individuals, but randomized controlled trials addressing the causal nature of such relationships are less clear. Preliminary evidence suggests a role of *n*-3 PUFA intake in mood and cognition in healthy individuals, particularly in times of stress. Using a double-blind, placebo-controlled design, 72 young adults were randomized to receive 2800 mg/day fish oil (n=36, 23 female, 20.8±2.4 years) or olive oil control (n=36, 22 female, 20.5±1.7 years) for 35 days. Subjects completed measures of mood, emotion regulation, and emotion-related cognitive processing following an acute stressor or non-stressful control task. 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.

ACKNOWLEDGEMENTS

I wish to thank members of the thesis committee: Robin B. Kanarek, Ph.D., Caroline R. Mahoney, Ph.D., and Heather Urry, Ph.D. Their expertise was paramount in completing this thesis. I especially thank my adviser, Robin Kanarek, and my mentor, Caroline Mahoney, for their knowledge, direction and support. Additional thanks to Tad Brunyé, Ph.D. for his continual guidance.

The work of research associates Jackie Hayes, Sarah Schuback, Sarah Tower-Richardi and Winnie Zhuang is greatly appreciated.

Thank you also to Patrick Sedney and my family, who have provided immense encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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

INTRODUCTION

Subtle changes in nutritional status may have significant impact on emotional and cognitive well-being. One such change may be polyunsaturated fatty acid (PUFA) levels, which play a role in a neurological and psychological function (Dacks, Shineman, & Fillit, 2013; Parker et al., 2006). Although much of the research on health benefits of polyunsaturated fatty acid intake stems from potential benefit to cardiovascular health and longevity (Djousse, Akinkuolie, Wu, Ding, & Gaziano, 2012; Hooper et al., 2006; Kotwal, Jun, Sullivan, Perkovic, & Neal, 2012), research has begun to address is psychoactive impact as well.

Omega-3 polyunsaturated fatty acid synthesis and function

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 docohexaenoic acid (DHA) (Parker et al., 2006). In the nervous system, PUFAs are released via neurotransmitter stimulation and metabolized to active compounds including prostaglandins, thromoxone, leukotrienes, and others. These compounds 1) act as neuronal second messengers, 2) interact with G-protein coupled receptors on glial cells, thereby affecting neuromodulation and synaptic output, 3) affect cell migration, 4) moderate neurogenesis, and synaptogensis and 5) increase adenylate cyclase and protein kinase A, which mediate serotonin, norepinephrine, and dopamine receptors (Fontani, Corradeschi, Felici, Alfatti, Bugarini et al., 2005a).

Current western diet is comprised of significantly greater *n-6* to *n-3* ratio than hunter-gatherer ancestors (between 17:1 to 10:1 compared to 1:1) due to increased intake of *n-6* PUFAs at the expense of decreased intake of *n-3* PUFAs (Heinrichs, 2010, Parker et al., 2006). In recent

years, the western diet has shifted from consumption of *n*-3 PUFA-rich foods such as fish, fish oil, wild game, wheat germ, walnuts and plants to saturated fat from domestic animals and *n-6* PUFAs from common vegetable oils, including corn, safflower, sunflower and soybean oil (Kiecolt-Glaser, 2010; Parker et al., 2006). Both *n*-3 and *n*-6 PUFAs are necessary for cells to maintain normal structure, function, and signal transduction. However, the ratio of *n*-6 to *n*-3 PUFAs, rather than the absolute levels is most important to these cellular processes (Simopoulos, 2011). For example, the ratio of *n*-6 to *n*-3 PUFA determines whether they will have inflammatory or anti-inflammatory actions. *n*-6 PUFA intake increases inflammation, whereas *n*-3 PUFAs reduce *n*-6 PUFA activity, and thus reduce inflammation (Calder, 2009; Calder, 2010).

Omega-3 PUFAs and mood

Such inflammatory processes may play a role in mood including mood disorders (Sinclair, Begg, Mathai, & Weisinger, 2007). A large body of research has investigated the potential antidepressant effects of *n*-3 PUFA supplementation, and extant evidence is generally split between studies finding a beneficial influence of *n*-3 PUFAs on depressive symptoms in individuals with major depressive disorder and those reporting null results (for reviews, see Giles, Mahoney, & Kanarek, 2013; Lin, Huang, & Su, 2010; Martins, 2009; Parker et al., 2006).

Epidemiological studies

Within the general population, multiple epidemiological studies have evaluated the correlation between *n*-3 PUFA intake and depressive symptoms across a number of geographical regions. *n*-3 PUFA intake was generally measured using food frequency questionnaires that assessed frequency of consumption as well as portion size of a range of food items including fish

and other types of seafood. Of the twelve studies assessing the relationship between *n*-3 PUFA intake and depressive symptoms in the general population, eight studies found inverse associations between fish or *n*-3 PUFA intake and depressive symptoms (see Tables 1 and 2 for further details). However, cross-sectional and prospective studies show correlations but not causation, especially given that most findings are based on self-report measures of PUFA consumption rather than including plasma or erythrocyte PUFA measures, which could validate reports of habitual consumption on food frequency questionnaires. Plasma or serum PUFA levels generally reflect acute intake and erythrocyte levels reflect long-term intake. Although both plasma and erythrocyte PUFA levels are associated with intake reported on food frequency questionnaires, erythrocyte levels are more strongly correlated (Sun, Ma, Campos, Hankinson, & Hu, 2007). Erythrocyte levels may be an efficacious measure to evaluate the association between PUFA levels and depressive symptoms. Thus extant research evaluating how *n*-3 PUFA and fish intake may relate to mood within the general population is fairly equivocal. Despite evidence that *n*-3 PUFA intake varies inversely with depressive symptoms in a number of populations, the majority of epidemiological lack biological markers to validate self reported PUFA intake, and such studies are correlational by definition and thus cannot be used to draw causal conclusions.

Randomized controlled trials

Such methodological limitations epidemiological studies reveal the need for randomized controlled trials to determine whether *n*-3 PUFA supplementation influences mood. The majority of trials assessing a potential causal relationship between *n*-3 PUFAs and mood have focused on individuals with major depressive disorder (Table 3), although a handful of studies have also looked at healthy individuals (Table 4). Six such trials in individuals with major depressive

disorder found beneficial effects of *n*-3 PUFA supplementation relative to placebo on depressive symptoms in men and women with major depressive disorder (da Silva et al., 2008; Lesperance et al., 2011; Lucas, Asselin, Merette, Poulin, & Dodin, 2009; Nemets, Stahl, & Belmaker, 2002; Peet & Horrobin, 2002; Su, Huang, Chiu, & Shen, 2003). On the other hand, seven studies found no influence of *n*-3 PUFA supplementation on depressive symptoms in men and women with major depressive disorder (Carney et al., 2009; Grenyer et al., 2007; Marangell et al., 2003; Rogers et al., 2008; Silvers, Woolley, Hamilton, Watts, & Watson, 2005).

Grenyer *et al.* (2007) evaluated the influence of the addition of 3 g/day *n*-3 PUFAs (approximately 1:4 EPA:DHA) to conventional psychotherapy (all subjects) and antidepressant medications (74% of subjects). Although no differences in depressive mood were found between the *n*-3 PUFA and placebo groups, the authors note a potential ceiling effect, wherein the combination of *n*-3 PUFA supplementation, psychotherapy, and pharmacological treatment may have masked beneficial effects of *n*-3 PUFA intake alone (Grenyer et al., 2007). Indeed, in a subsequent study by the same group, Meyer *et al.* (2013) assessed the influence of 8 g/day DHA-rich tuna oil containing 2.6 g *n*-3 PUFA (approximately 1:3.3 EPA:DHA) on depressive symptoms (Meyer et al., 2013). Although changes in depression did not differ between individuals supplemented with DHA-rich tuna oil and with the olive oil control, erythrocyte DHA was inversely associated with depressive symptoms. These results point again to the need to include relevant biological markers including erythrocyte PUFA levels in order to better understand the relationship between PUFA intake and mood, and highlight the possibility that studies looking only at between-group differences (i.e. *n*-3 PUFA versus placebo supplementation) may fail to detect whether changes in PUFA levels predict changes in mood.

A few published empirical studies have assessed the effect of *n*-3 levels and supplementation on mood and emotion regulation 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; Fontani, Corradeschi, Felici, Alfatti, Migliorini et al., 2005b). 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, Van der Does, Smelt, & Rogers, 2009).

Thus placebo-controlled trials assessing mood in healthy individuals are limited, but given that subclinical depressive symptoms vary along a continuum in otherwise healthy individuals (Kessler, Zhao, Blazer, & Swartz, 1997) more research in this population is needed to explore the effects.

Omega-3 PUFAs, Mood, and Stress

Stress may be one pathway by which *n*-3 PUFA levels modulates mood. 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).

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS), stimulating adrenocorticotropic hormone (ACTH) and producing elevations in glucorticoids, cortisol, alpha-amylase, and heart rate (Gerra et al., 2001; Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004; Raedler, 2011). ACTH is elevated in individuals with acute stress, chronic stress, and major depression (Raedler, 2011). Stress and depression increase the

risk of infection, prolong infectious episodes, and delay wound healing, all of which enhance proinflammatory cytokine production. Cytokines are a broad class of polypeptide mediators that are secreted by cells of the body, often as part of an immune response. Cytokines may be either proinflammatory, including interleukin (IL)-1, IL-6, tumour necrosis factor (TNF) which augment the immune response or they may be anti-inflammatory, including IL-4, IL-10, and IL-13 which diminish the immune response (O'Brien, Scott, & Dinan, 2004).

Dietary *n*-6 PUFA intake increases the production of proinflammatory cytokines including IL-1, tumor necrosis factor (TNF)-α, and IL-6. *n*-3 PUFA intake reduces the activity of *n*-6 PUFAs, thereby reducing proinflammatory cytokine activity (Calder et al., 2009; Calder, 2010). A recent study evaluated the effects of three months of *n*-3 PUFA supplementation on depression, anxiety, and proinflammatory cytokines IL-6 and (TNF)-α in healthy individuals (Kiecolt-Glaser, Belury, Andridge, Malarkey, & Glaser, 2011). Results indicated that *n*-3 PUFA supplementation significantly decreased IL-6 and anxiety and marginally decreased (TNF)-α. Although supplementation had no influence on depressive symptoms, plasma *n*-3 PUFA levels were inversely associated with anxiety, and the *n*-6 to *n*-3 ratio was directly associated with anxiety and cytokine production.

The ratio of *n*-6 to *n*-3 PUFA may influence physiological stress responses akin to those observed in depression in healthy individuals as well. Indeed, Delarue *et al.* (2003) examined the effects of 1.1 and 0.7 g/day *n*-3 PUFA supplementation on adrenal activation following an experimentally-induced stressor in seven healthy men. Acute stress increased heart rate, blood pressure, plasma epinephrine, norepinephrine, cortisol, non-esterified PUFAs, and oxygen consumption. Three weeks of *n*-3 PUFA supplementation eliminated stress-induced cortisol increase and dampened increases in epinephrine. No differences were seen in the stress-induced

changes to heart rate, blood pressure, and norepinephrine. Blunted cortisol and epinephrine response to stress indicates that *n*-3 PUFA intake reduced adrenal activation in response to stress whereas lack of change in heart rate suggests that *n*-3 PUFA supplementation did not affect activation of the sympathetic nervous system (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 also influence stress-induced changes to mood in healthy individuals.

Omega-3 PUFAs and cognitive function

Despite the benefits of *n*-3 PUFAs when brain functions go awry, as in psychological and neurological disorders (Dacks et al., 2013; Parker et al., 2006) less research has examined the influence of *n*-3 PUFAs on cognitive function in healthy individuals. Recent research has begun to fill this gap, by examining how *n*-3 PUFA supplementation influences cognitive development in infants and children, cognitive performance in young adults, and age-related cognitive impairment in older adults. Just as the brain changes throughout the lifespan, so too may nutritional influences on cognition (Benton, 2010; Luchtman & Song, 2013).

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 (see Tables 5 and 6 for details).

Cognitive function in young adults

To date only a handful of studies have assessed the cognitive effects of *n*-3 PUFA supplementation in young adults (Table 7). Among the few, Fontani *et al.* (2005b) found that five weeks of 4 g/day fish oil (1.6 g EPA, 0.8 g DHA) reduced reaction time on tasks of response

inhibition (Go/No-Go) and sustained attention (complex Go/No-Go) (Fontani, et al. 2005b). However, fish oil did not influence simple or choice reaction time tasks. Thus, evidence from Fontani and colleagues suggests that *n*-3 PUFAs have beneficial effects on mood as described earlier, but does not present a clear picture on common cognitive tasks.

More recent data showed that four weeks of 3 g/day fish oil (1.74 g EPA, 0.25 g DHA) supplementation did not influence cognitive performance across a range of tasks measuring response inhibition, facial expression recognition, and memory (Antypa et al., 2009). Fish oil increased risk-seeking decision-making in gains only trials of a gambling task as well as reaction time in both gains-only and losses-only trials relative to placebo, together suggesting that *n*-3 PUFA supplementation increased willingness to make calculated risks rather than increased impulsiveness. However, the risk-based decision making task was only administered after supplementation, making it impossible to determine whether *n-3* PUFA and control supplementation differentially changed risk taking pre- to post-supplementation. Another study found that 4 weeks of 1 g/day fish oil (.36 g EPA, .24 g DHA) supplementation improved verbal learning, but did not influence response inhibition or mood (Karr, Grindstaff, & Alexander, 2012). Even more recently, 24 weeks of 1.16 g/day DHA supplementation improved episodic memory in women and working memory in men, but not attention or processing speed (Stonehouse et al., 2013).

Additional evidence that *n*-3 PUFA intake may influence neural function comes from a study in healthy 8-10 year old boys, in which two doses of DHA (.4 or 1.2 g/day) 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. Previous neuroimaging studies indicate hypo-activation in the

DLPFC in individuals with depression compared to healthy controls (for review, see Koenigs & Grafman, 2009). The ventromedial prefrontal cortex (VMPFC), which is hyperactive in individuals with depression, is generally thought of as the "emotional" division of the prefrontal cortex whereas the DLPFC is thought of as the "cognitive" division. However evidence now suggests that the DLPFC shares emotional functions as well, specifically, through cognitive reappraisal and suppression (Koenigs & Grafman, 2009). To our knowledge the only study to assess the influence of *n*-3 PUFA intake on functional brain activation is that of McNamara and colleagues (2010). Given that they found up regulation of DLPFC activity and that depression is associated with reduced DLPFC activation, it is plausible that *n*-3 PUFA supplementation may influence DLPFC-associated cognitive function, including mood and emotion-related cognitive processing.

Omega-3s, stress, and cognition

To date, all available evidence concerning the influence of *n*-3 PUFA on stress-induced changes in cognition come from animal studies, which suggest that *n-3* fatty acids overcome stress-induced 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). Additionally *n-3* PUFA-enriched diet fed to rats with traumatic brain injury normalized brain-derived neurotropic factor levels, reduced oxidative damage, and improved water maze performance, which reflects spatial learning and memory. Multiple animal studies suggest that *n-3* deficient diet is associated with learning deficits and heightened anxiety, suggesting that *n-3* PUFA deficiency impedes learning and memory and may have anxiogenic properties (Heinrichs, 2010). Ferraz and colleagues (2011) assessed the interactive effects of *n*-3

PUFA supplementation and stress on anxiety, depressive-like behavior, and spatial reference memory in rats, finding that *n-3* PUFA supplementation prevented anxiety- and depressive-like behaviors and learning and memory deficits induced by stress. Further, stress increased corticosterone but fish oil supplementation blunted this increase to levels of the non-stressed groups. Plasma corticosterone levels were associated with anxiety-like behavior (Ferraz et al., 2011). Similarly, Trofimiuk and Braszko (2011) found that cod liver oil (300 mg DHA, 225 mg EPA) reduced restraint stress-induced impairment on recall and spatial memory (Trofimiuk & Braszko, 2011).

The present study

Preliminary evidence exists for anti-depressive actions of *n-3* PUFA (for reviews, 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). It is also known that stress and depression as well as high *n-6* to *n-3* PUFA ratio increase sympathetic hyperactivity, oxidative stress, and proinflammatory cytokine production. 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 reduced intake of *n-3* PUFAs, these questions warrant further empirical study.

The primary objective of the present study was to evaluate the influence of *n*-3 PUFA supplementation on stress-induced changes in mood. We chose the Profile of Mood States (POMS; McNair, Lorr, & Droppleman, 1971) and State–Trait Inventory for Cognitive and Somatic Anxiety (STICSA; Ree, French, MacLeod, & Locke, 2008) based on previous studies

using similar doses (i.e. 1.6 g/day EPA plus 0.8 g/day DHA for five weeks). These studies found increased rated vigor and reduced rated anger, anxiety, fatigue, depression, and confusion on the POMS (Fontani, et al. 2005a,b) and a lower dose (0.2 g/day EPA plus 0.4 g/day DHA) reduced rated anxiety on the Beck Anxiety Inventory (Antypa et al., 2009). However, we selected the STICSA in place of the Beck Anxiety Inventory in order to better understand if and how *n*-3 PUFA supplementation differentially affects the cognitive and somatic dimensions of anxiety. The STICSA has been shown better reflect anxiety than depression symptoms relative to the more commonly-used State-Trait Anxiety Inventory (Gros, Antony, Simms, & McCabe, 2007). 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 stress would impair mood but that *n*-3 PUFA supplementation would reduce this effect 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. 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 three tasks to evaluate a range of such processing, including the Emotional Interference Task (EIT; Dolcos & McCarthy, 2006), Morphed Faces Task (MFT; Brunyé, Howe, & Mahoney, 2013; Joormann & Gotlib, 2006; Young et al., 1997), and the Cognitive Reappraisal Task (CRT; Jackson, Malmstadt, Larson, & Davidson, 2000). Such tasks have been shown to activate brain regions similar to those influenced by *n*-3 PUFA supplementation, including prefrontal regions such as the DLPFC (Dolcos & McCarthy, 2006; Ochsner, Bunge, Gross, & Gabrieli, 2002). Given that DHA supplementation enhanced prefrontal activation associated with

cognitive processing, we hypothesized that *n*-3 PUFA supplementation would enhance performance on these tasks.

Because *n*-3 PUFA supplementation has been shown to influence physiological changes following stress (Delarue et al., 2003), and because inflammation may be one pathway by which *n*-3 PUFA intake influences mood (Kiecolt-Glaser, 2010; Sinclair et al., 2007), the present study also sought to quantify the interactive effects of *n*-3 PUFA and stress on salivary cortisol, a biomarker of arousal (Hellhammer & Schubert, 2012) and the proinflammatory cytokine interleukin 1β (IL-Iβ), a biomarker of inflammation (Calder, 2010). We expected that stress would increase both salivary cortisol and IL-Iβ, but that this effect would be less pronounced following *n*-3 PUFA than control supplementation.

METHODS

Seventy two students (FO: 13 male, 23 female, mean age 20.80±2.39, mean BMI 20.45±0.73; OO: 14 male, 22 female, mean age 20.49±1.70, mean BMI 21.67±0.73) participated for monetary compensation. Target sample size of 72 was based on previous evidence that found significant mood and cognitive effects following *n*-3 PUFA supplementation with 33 (Fontani, et al. 2005a), 36 (Fontani, et al. 2005b) and 54 subjects (Antypa et al., 2009). Five additional students began the study but withdrew from participation due to stomach upset (n=4 FO, n=1 OO). All 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 with Treatment (5 weeks 2800 mg/day fish

oil “FO”, 2800 mg/day olive oil control “OO”) as the between-subjects factor and Stress (stress; no stress) and Time (pre-/post-supplementation) as within-subjects factors.

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). All capsules appeared identical in shape and size. Dose levels were chosen that are within the range of:

(1) Doses used in previously published omega-3 fatty acid work, e.g. 2.3 g *n*-3 PUFA (Antypa et al., 2009) and 2.8 g *n*-3 PUFA (Fontani, et al. 2005b).

(2) Doses readily available in commercial products in retail stores such as GNC (i.e. Nordic Naturals, Inc. OMEGA-3 Purified Fish Oil; 1725 mg) and CVS (i.e. Nature's Bounty Omega-3 Fish Oil; 1200 mg).

(3) Amounts consumed in other parts of the world. Omega-3 consumption in Japan averages 4 g/day (Sugano, 1996). Median omega-3 intake of Alaskan Eskimo men: 3 g (17-39 years), 4.3 (40-60 years), 4.0 (61-92 years) and women: 2.8 g (17-39 years), 3.5 (40-60 years), 3.4 (61-92 years) (Nobmann et al., 2005).

Manipulation checks

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. Such foods included fish (salmon, sardines, tuna, cod, mackerel, swordfish, crab, lobster, bluefish, smelt, scallops), nuts and seeds (almonds, walnuts, flax seed, pecans, pistachios, poppy seeds, pumpkin seeds, sesame seeds),

oils (walnut oil, soybean oil, flax seed oil, canola oil, cod liver oil, olive oil, sardine oil), grains and beans (soybean oil, tofu) and greens (spinach, kale, collard greens) (Tufts University School of Medicine, Nutrition Infection Unit, 2002). One serving size equaled 4 oz (fish and tofu) 1 oz (nuts), 1 tablespoon (oils), and ½ cup (soybeans and greens). Subjects were asked to report the number of servings of each food they had eaten in the past week, and were given examples of serving sizes (e.g. 4 oz equals a bar of soap; Fitsugar, 2007) for reference FO and OO groups did not differ in *n*-3 (p=.445) or *n*-6 (p=.143) intake at baseline, or during any of the five study weeks (see Table 9).

Text message reminders. Participants consumed seven capsules every day for five weeks. They came to the lab every weekday and consumed the capsules in front of an experimenter. The experimenter sent text messages to the participants on Saturdays and Sundays, reminding them to take their capsules. Participants were required to reply to confirm that they took their capsules. 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 compliance rates of 99.60% in the FO group and 99.29% in the OO group.

Mood and physiological measures

In order to assess the effect of FO supplementation on stress-induced mood and physiological changes, participants completed the Profile of Mood States (POMS) Questionnaire and State–Trait Inventory for Cognitive and Somatic Anxiety (STICSA). Heart rate was collected continuously throughout each test session. Saliva was taken to measure cortisol and the pro-inflammatory cytokine interleukin-1β (IL-1β).

Profile of Mood States Questionnaire (POMS). The POMS is an inventory of subjective mood and arousal states (McNair et al., 1971). Participants were asked to rate a series of 65 mood-related adjectives on a five point scale, using the response set of "How are you feeling right now?" Previous research has shown that the adjectives factor into six subscales; tension, depression, anger, vigor, fatigue, and confusion as well as a composite score of total mood disturbance (Lieberman, Mays, Shukitt-Hale, Chinn, & Tharion, 1996).

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, which participants rate on a 4-point Likert scale, ranging from 1 (not at all) to 4 (very much so) (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). The STICSA State assesses how participants "feel right now, at this very moment, even if this is not how you usually feel." The STICSA Trait asks participants "how often, in general, the statement is true of you."

Emotion regulation questionnaire (ERQ). The ERQ is comprised of 2 scales, the reappraisal scale and suppression scale, which ask the participants to rate their behavior during the stressful or non-stressful situation on a 6-point Likert scale, ranging from 0 (not at all) to 5 (extremely) (Egloff, Schmukle, Burns, & Schwerdtfeger, 2006). The reappraisal scale consisted of: "I tried to see the situation as positive as possible," "I viewed the situation as a challenge," and "I thought of the situation in a way that made me stay calm." The suppression scale was composed of "During the situation, I controlled my emotions," "I showed my emotions," and "One could see my feelings during the situation."

Heart rate. Heart rate data was collected using an Equivital heart rate monitor. The monitor consisted of a transmitter worn against the skin and around the chest. The transmitters picked up and stored temporarily signals from the participant's heart and skin. The data was downloaded at the end of each experimental session. Participants were instructed on the proper placement of the heart rate strap and then asked to don the strap and sensor themselves. The experimenter then confirmed the signal.

Saliva. Saliva was collected for analyses of salivary cortisol and the pro-inflammatory cytokines interleukin-1β (IL-1β). Participants were instructed to spit through a straw into a 15 ml saliva collection tube. They were asked to fill the tube half-way and to avoid touching the mouth of the tube with their hands. Samples were aliquoted into 2-1.8 ml plastic vials. The samples were stored at -20°C until they were assayed. Samples were analyzed in duplicate in an independent laboratory (Salimetrics LLC, State College, Pennsylvania).

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, Pirke, & Hellhammer, 1993) . In the first stage, participants were led into a conference room and introduced to a panel of three experimenters. They were given 10 minutes to prepare a 5-minute mock job-talk that would be videotaped and assessed for nonverbal behavior and voice frequency. In the second stage, participants delivered the 5-minute speech. If they ended in less than 5 minutes, they were asked to continue talking. In the third stage, participants completed a mental arithmetic task, in which they serially subtracted a prime number from a 4-digit number (e.g. 17 from 1223) and had to

start over if they made a mistake. The control condition consisted of a 5 min 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, Piel, & Wolf, 2005).

Cognitive tasks

Three cognitive tasks were administered at 3 time points throughout the study; Week 1 prior to first supplementation (Day 0) and twice at the end of week 5 (Days 34 and 35), immediately following the stressful and non-stressful TSST (counterbalanced across participants).

Emotional Interference Task (EIT). The EIT task is a modified Sternberg item recognition paradigm using visuospatial stimuli of abstract shapes and/or neutral-expression faces (Erk, Kleczar, & Walter, 2007; Wolf & Walter, 2005), which measures attentional control and the ability to regulate emotion. Participants completed a delayed match-to-sample task, with distracters presented during the delay period (Dolcos & McCarthy, 2006). During each trial, three visuospatial stimuli were presented during the initial stimulus presentation, and then two scrambled, neutral or negative distracter images were presented immediately prior to a recognition task. Distracter images were selected from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 2005) and Geneva Affective Picture System (GAPED; Dan-Glauser & Scherer, 2011). Dependent measures include accuracy and response time.

Morphed Faces Task (MFT): The MFT involves an adapted task to display the dynamic onset and offset of six facial emotions: anger, disgust, happiness, fear, sadness, and surprise (i.e.,

Brunyé et al., 2013; Joormann & 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 the face displayed the target emotion. 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. A total of 8 faces, each displaying the six emotions were used (for a total of 72 trials). Half of the trials depicted emotional expression onset, and half offset. Dependent measures included (1) 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 that lower stop frames signify higher sensitivity to the target emotion.

Cognitive Reappraisal Task (CRT). The CRT was administered post-supplementation (i.e. Days 34 and 35) only. The CRT involves viewing a series of unpleasant images from the IAPS and GAPED (Dan-Glauser & Scherer, 2011; Lang et al., 2005) while attempting to either reappraise or maintain their thoughts of the images. While viewing each image, participants were trained to follow one of two auditory instructions during each picture trial: suppress or maintain (Jackson et al., 2000; Urry, 2009). When participants heard the word "suppress," they were asked to imagine the situation improving, whereas when they heard the word "maintain," they were asked to imagine the situation staying the same and avoid attempting to change their thoughts toward the picture. They were then asked to rate the picture's unpleasantness a scale ranging from 1 (not at all unpleasant) to 7 (very unpleasant). In order to confirm that participants understood the task, practice trials were administered, after which the investigator asked subjects to explain their though processes while hearing the suppress and maintain instructions. A total of

48 trials of unpleasant images were presented, half of which included the instruction to suppress (n=24) and half to maintain (n=24).

Procedure

Participants completed four sessions on separate days: one practice session 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). During the practice session, participants completed screening materials and signed the informed consent. They were then familiarized with test procedures, including putting on the heart rate monitor and questionnaires. They completed trait and state measures for mood including the POMS and STICSA. They then received instructions for the cognitive tasks and completed practice trials. In addition, height and weight were taken. 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 donned the heart rate strap and completed baseline measures of the POMS and STICSA-S and salivary cortisol. During the first test session, participants then completed the Emotional Interference Task and Morphed Faces Task tasks, followed by a second set of questionnaires and salivary cortisol and IL-1β. Participants consumed FO or OO every day for 35 days. To promote treatment adherence, participants were required to come to the lab each weekday and take 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. The final two test sessions took place on the 34^{th} and 35^{th} day of supplementation. Following the baseline POMS, STICSA-S and salivary cortisol measurements in the final two sessions, participants completed either the stressful or non-

stressful TSST. Participants then completed a second set of questionnaires and saliva sample. They completed a battery of cognitive tasks, which include the Emotional Interference Task, Morphed Faces Task and Cognitive Reappraisal Task, in that order (see Figure 1 for schematic of procedure).

Baseline Group Differences

First, to determine whether the FO and OO groups differed on any measures prior to supplementation, all measures were subjected to univariate analysis of variance (ANOVA)s with supplementation 2(FO, OO) as the between-subjects factor and alpha levels corrected using the Bonferroni correction. The two POMS and STICSA time-points pre-supplementation were averaged and then compared. No single comparison reached full or marginal significance, with the exception of the cognitive anxiety subscale of the STICSA-S, in which rated state cognitive anxiety was higher in the FO than OO group. For means and standard deviations, see Table 9.

Statistical Methods

Change scores were calculated for each POMS and STICSA subscale on both the stress- and control-TSST sessions (Post-TSST – Pre-TSST), such that positive values indicate an increase from baseline and negative values indicated a decrease from baseline. All measures 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. Exploratory analyses also included gender 2(Male, Female) as a between subject factor. Unless otherwise noted (see salivary cortisol and Morphed Faces Task), no gender differences were found (all *p*s>.13).

Because similar studies found that salivary cortisol concentrations had skewed distributions (Schoofs, Preuss, & Wolf, 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.

An effect was deemed statistically significant if the likelihood of its occurrence by chance was $p<0.05$. When sphericity was violated, Greenhouse–Geisser corrected *p*-values were used. When an ANOVA yielded a significant main effect, post-hoc tests using the Bonferroni correction were conducted. All statistical analyses were performed using SPSS 12.0.

RESULTS

Demographic Information

Table 10 shows demographic information for the FO and OO groups. Groups did not differ on any of the measures (all *p*s > .140).

Blinding and Side Effects

Of the 36 subjects in each group, 30 (83%) subjects in the FO and 16 (44%) subjects in the OO group correctly guessed their treatment assignment. Thus, subjects given FO were able to guess their treatment group above chance. Side effects included fishy burps and aftertaste (n=6 FO), queasiness, upset stomach and bloating (n=1 FO, 4 OO), loose or discolored stools (n=2 OO) and headaches (n=1 FO, 1 OO).

Mood Questionnaires

POMS. POMS data reflects 71 subjects (n=36 FO, 35 OO) as one subject failed to complete the POMS (Table 11).

Tension. Analysis of the tension subscale revealed a main effect of Stress $F(1,70)=33.468$, $p<.001$ ($\eta^2=.239$) in which feelings of tension increased during the stressful TSST but decreased during the non-stressful TSST (Stress=3.65±.68, Non-Stress=-1.03±.44). No effects were found for diet Group ($p=.875$) or Stress x Group ($p=.663$).

Depression. Analysis of the depression subscale revealed a main effect of Stress $F(1,70)=6.838$, $p=<.05$ ($\eta^2=.049$) in which feelings of depression increased during the stressful TSST but decreased during the non-stressful TSST (Stress=.41±.59, Control=-1.28±.46). No effects were found for diet Group ($p>.18$) or Stress x Group ($p>.49$).

Anger. Analysis of the anger subscale revealed a main effect of Stress $F(1,70)=10.865$, $p<.01$ ($\eta^2=.095$) in which feelings of anger increased during the stressful TSST but decreased during the non-stressful TSST (Stress=2.04±.75, Control=-.89±.42). A main effect of diet Group $F(1,70)=1.949$, $p<.05$ ($\eta^2=.018$) showed that feelings of anger remained stable in the FO group but increased in the OO group (FO=-.34±.57, OO=1.49±.59). No effect was found for Stress x Group ($p>.34$).

Confusion. Analysis of the confusion subscale revealed a main effect of Stress $F(1,70)=17.817$, $p<.001$ ($\eta^2=.150$) in which feelings of confusion increased during the stressful TSST but decreased during the non-stressful TSST (Stress=1.98±.48, Control=-.59±.31). A Stress x Group interaction $F(1,70)=4.345$, $p<.05$ ($\eta^2=.037$) showed that feelings of confusion increased during the stressful TSST and decreased during the non-stressful TSST in the OO

group $t(34)=-4.039$, $p<.001$ but remained relatively stable in the FO ($p=.100$) group (Figure 2). No main effect was found for diet Group ($p>.94$).

Total Mood Disturbance. Analysis of the total mood disturbance composite score revealed a main effect of Stress $F(1,70)=15.586$, $p<.001$ ($\eta^2=.132$) in which total mood disturbance increased during the stressful TSST but decreased during the non-stressful TSST (Stress=6.87±2.39, Non-Stress=-5.06±1.70). No effects were found for diet Group ($p>.27$) or Stress x Group ($p>.38$).

Vigor. Within the vigor subscale, no effects were found for Stress ($p>.71$), diet Group ($p>.46$) or Stress x Group ($p>.38$).

Fatigue. Within the fatigue subscale, no effects were found for Stress ($p>.49$), diet Group ($p>.41$) or Stress x Group ($p>.44$).

STICSA

Somatic Anxiety .Analysis of rated somatic anxiety revealed a main effect of Stress $F(1,70)=14.989$, $p<.001$ ($\eta^2=.108$) in which somatic anxiety increased during the stressful TSST and decreased during the non-stressful TSST (Stress=0.67±0.29, Non-Stress=-0.78±0.27; Table 13). A Stress x Group interaction $F(1,70)=8.004$, $p<.01$ ($\eta^2=.058$) showed that rated somatic anxiety was lower in the non-stressful than stressful TSST in the FO $t(35)=-4.399$, $p<.001$ but not OO ($p>.42$) group (Figure 3). No main effect was found for diet Group ($p>.11$).

Cognitive Anxiety. Analysis of rated cognitive anxiety revealed a main effect of Stress $F(1,70)=4.924$, $p<.05$ ($\eta^2=.045$) in which cognitive anxiety increased during the stressful TSST but decreased during the non-stressful TSST (Stress=0.46±0.49, Non-Stress=-0.78±0.26). No effects were found for diet Group ($p>.94$) or Stress x Group ($p>.38$).

Emotion Regulation Questionnaire. Analysis if the Emotion Regulation Questionnaire revealed a main effect of Stress $F(1,70)=8.189$, $p<.01(\eta^2=.030)$ and Strategy x Stress interaction $F(1,70)= 17.418$, $p<.001$ ($\eta^2=.051$) (Table 13). These effects were qualified by higher use of reappraisal than suppression during the stressful $F(1,70)=16.840$, $p<.001$ but not non-stressful TSST ($p>.19$; Figure 4).

Physiological measures

Heart rate. Heart rate data was limited to 37 subjects (n=16 FO, n=21 OO) due to monitor malfunction. In order to confirm that heart rate was higher during the stressful than non-stressful TSST and evaluate whether it differed as a function of FO or OO supplementation, heart rate data (in beats per minute; bpm) was divided into three time 20-minute periods: pre-TSST (baseline), TSST and post-TSST. Heart rate was then analyzed using a repeated-measures ANOVA with stress 2(Stress, Non-Stress) and time 3(pre-TSST, TSST, post-TSST) as the within-subjects factors and Group (FO, OO) as the between subjects factor (Table 14). A main effect of Time $F(2,70)=47.300$, $p<.001$ ($\eta^2=.144$) showed that heart rate was higher than baseline during the TSST and lower than baseline post-TSST (pre-TSST=76.28±1.71, TSST=78.43±1.63, post-TSST=71.22±1.62). A Time x Stress interaction $F(2,70)=12.425$, $p<.001$ ($\eta^2=.024$) showed that heart rate was higher during the stressful than non-stressful TSST $F(1,35)=8.334$, $p=<.01$ but not before ($p>.98$) or after ($p>.35$) the TSST (Figure 5). No effects were found for diet Group ($p>.96$), Time x Group ($p>.96$), Stress ($p>.14$), Stress x Group ($p>0.64$) or Stress x Time x Group ($p>.72$).

Cortisol. Cortisol data reflects 71 subjects (n=36 FO, 35 OO) as one subject failed to provide sufficient saliva (Table 15). Analyses were performed on log transformed data but raw

data (μg/dL) are reported. A main effect of Stress showed that cortisol was higher during the stressful than non-stressful test session $F(1,69)=9.803$, $p<.01$ ($\eta^2=.024$) (Stress = .38±.03, Non-Stress=.32±.02). A main effect of Time showed that cortisol was higher 10-minutes after the TSST than before or 60-minutes after the TSST $F(2,138)=33.653$, $p<.001$ ($\eta^2=.154$) (Pre-TSST = .37±.03, 10 minutes post-TSST = .40±.02, 60 minutes post-TSST = .26±.02). A main effect of Gender $F(1,67)=9.614$, $p<.01$ ($\eta^2=.024$) showed that cortisol levels were higher in females than males (Female=.40±.03, Male=.27±.06). No differences were found for diet Group ($p>.10$), Stress x Time ($p>.10$), Stress x Group ($p>.49$), Time x Group ($p>.07$), or Stress x Time x Group ($p>.85$).

IL-1β. A main effect of stress showed that IL-Iβ (in pg/mL) was higher during the stressful than non-stressful test session $F(1,70)=4.140$, $p<.05$ ($\eta^2=.011$) (Stress=190.96±24.09, Non-Stress=227.27±.74.22; Table 16). No effects were found for diet Group ($p>.96$) or Stress x Group (p>.19). Analyses were performed on log transformed data but raw data are shown reported.

Cognitive tasks

EIT. Analysis of accuracy showed a main effect of Stress $F(1,70)=5.117$, $p<.05$ ($\eta^2=.013$) in which accuracy was lower following the stressful than non-stressful TSST (Stress=0.88±0.02, Control=0.91±0.01; Table 15). No other main effects or interactions were found for accuracy or response time (all *p*s>.2).

Morphed Faces Task. Analysis of rated intensity showed a main effect of Emotion $F(5,340)=99.044$, $p<.001$ ($\eta^2=.523$) such that angry faces were rated as most intense, followed by (in order from most to least intense) fear, happy, disgust, surprise, and sad (Anger=5.09±0.10,

Fear=4.35±0.10, Happy=3.00±0.18, Disgust=2.90±0.17, Surprise=2.84±0.16, Sad=2.62±0.10; Table 16). An Emotion by Stress by Gender interaction $F(5,330)=3.838$, $p<.01$ ($\eta^2=.002$) revealed that for happy faces $F(1,66)=14.253$, $p<.001$ females rated happy faces are more intense following the stressful TSST ($p<.01$) whereas males rated happy faces as more stressful during the non-stressful ($p<.05$). No differences were found for other emotional expressions (all ps>.12).

Analysis of stop frames revealed a main effect of Emotion $F(5,350)=380.825$, $p<.001$ ($\eta^2=.835$). Stop frames were lowest (i.e. higher sensitivity to emotional expression) for disgust, followed by (in order from low to high stop frames) happy, surprise, anger, fear, and sad (Disgust=16.33±1.42, Happy=18.14±1.52, Surprise=24.11±1.66, Anger=68.24±1.89, Fear=77.23±1.83, Sad=87.09±1.24; Table 17). An Emotion by Gender interaction $F(5,340)=6.099$, $p<.001$ ($\eta^2=.012$) showed lower stop frames 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 other main effects or interactions were found (all ps>.30).

Cognitive Reappraisal Task. First, to verify that images were matched for normative pleasantness ratings, we used a univariate ANOVA with stress 2(Stress, Non-Stress), strategy 2(reappraise, maintain), and image 2(IAPS, GAPED) as within subjects factors. Because normative ratings for IAPS were assessed using the Self-Assessment Manikin (SAM) which is a 9 point scale (1=low pleasure and 9=high pleasure; (Lang et al., 2005) whereas those for GAPED were assessed on scale from 1-100 (1=very negative, 50=neutral, 100=very positive; (Dan-Glauser & Scherer, 2011), we divided GAPES images by 11.1 to roughly equate the two scales. This analyses revealed a main effects of Image $F(1,88)=74.835$, $p<.001$, in which IAPS images

were rated as more pleasant than GAPED images (IAPS=1.756±.053, GAPED=.526±.116). No effects were found for Strategy (p>.34), Stress (p>.91), Strategy x Stress (p>.43) or Strategy x Image (p>.852).

However, because scales used to ascertain normative ratings from IAPS and GAPES image differed so dramatically, to determine whether difference in image source overshadowed differences between stress and strategy conditions, normative ratings were also subjected to a univariate ANOVA with only stress 2(Stress, Non-Stress) and strategy 2(reappraise, maintain) as within subjects factors. Here, normative rated pleasantness was higher in reappraise than maintain condition $F(1,90)=5.069$, $p<.05$ (Reappraise=1.658±.088, Maintain=1.380±.086) and in the non-stressful than stressful TSST condition $F(1,90)=13.983$, $p<.001$ (Stress=1.289±.087, Non-Stress=1.750±.087). A Strategy x Stress interaction $F(1,90)=5.643$, $p<.05$ revealed that normative rated pleasantness was higher in the reappraise than maintain condition in the stressful TSST $F(1,45)=6.259$, $p<.05$ (Reappraise=1.574±.163, Maintain=1.003±.160) but not the non-stressful TSST (p>.87).

Thus, analyses were limited to the non-stressful TSST condition, in which normative rated pleasantness did not differ between images shown in reappraisal and maintain trials. Results showed a main effect of Strategy $F(1,70)=62.164$, $p<.001$ ($\eta^2=.208$) in which ratings of unpleasantness were lower during reappraisal than maintain trials (Reappraisal=4.114±.122, Maintain=4.877±.112), but no effects diet Group (p>.53) or Strategy x Group (p>.60; see Table 18).

DISCUSSION

We evaluated the influence of *n*-3 PUFA supplementation on stress-induced changes to mood and emotion-related cognitive processing. We found that the stress manipulation exerted effects consistent with extant stress literature, including impairing mood and anxiety and elevating heart rate, cortisol and IL-1β. However we detected limited effects of *n*-3 PUFA supplementation on mood, cognition, or physiological responses.

n-3 PUFA effects on mood

Stress impaired mood, including augmenting feelings of tension, depression, anger, confusion and cognitive and somatic anxiety. Rated anger and confusion increased with stress in the OO group relative to baseline, but remained stable in the FO group. Additionally, rated somatic anxiety was lower following the non-stressful than stressful Trier Social Stress Test following FO but not OO supplementation. Thus overall *n*-3 PUFA supplementation exerted limited effects on mood and do not support our hypothesis that that FO 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 symptoms within the general population are quite mixed. In Finland, Tanskanen *et al.* (2001) and Timonen *et al.* (2004) found an inverse relationship between fish intake and depressive symptoms in women only (Tanskanen et al., 2001; Timonen et al., 2004). Similar results were found for women only using depression, anxiety and stress diagnoses (Colangelo, He, Whooley, Daviglus, & Liu, 2009; Sanchez-Villegas et al., 2007). Astorg *et al.* (2008) found that moderate to high fish intake was negatively correlated with the risk of depressive episode, and this correlation was stronger in men than

women (Astorg et al., 2008). Other studies found beneficial effects in both men and women in the United Kingdom (Appleton et al., 2007) and in Greece (Panagiotakos et al., 2010). Lucas *et al.* (2009) assessed the association between various *n*-3 and *n*-6 PUFAs and depression in women. They found positive associations between the *n*-6 PUFA precursor linolenic acid (LA) intake and risk of clinical depression, as well negative associations between the between the ratio of *n*-3 precursor alpha linolenic acid (ALA) to *n-6* PUFA arachidonic acid (ARA) the risk of clinical depression (Lucas et al., 2009). In a subsequent publication, the same group prospectively studied women age 50 to 77 years old, initially free of depression. During the 10-year follow-up, risk of depression was positively associated with LA intake and *n*-6 to *n*-3 ratios. Risk of depression was negatively associated with ALA to LA ratios (Lucas et al., 2011).

On the other hand, a number of studies looking within the general population found no relationship between *n*-3 PUFA intake and depressive symptoms. In a sample of Finnish men, Hakkarain *et al.* (2004) found only trends toward increased risk for self-reported depressed mood in the highest and lowest tertiles of fish consumption (Hakkarainen et al., 2004). No relationship was found in a Japanese sample (Murakami et al., 2008). In a Taiwanese sample, no relationship was found between plasma fatty acid composition and Hamilton Depression Rating Scale (HDRS) scores (Jadoon et al., 2012). Appleton *et al.* (2008) examined fasting blood samples from a non-clinical population, finding no associations between plasma *n*-3 PUFA concentrations or *n*-6 to *n*-3 ratios and depression, even after controlling for variables such as age and gender (Appleton et al., 2008).

Similar to results from epidemiological studies, those from randomized controlled trials assessing the influence of PUFA supplementation on mood are inconsistent. Peet and Horrobin (2002) examined the influence of three doses of EPA on symptoms of depression and found that

1 g/day EPA improved all measures of depression, beginning 4 weeks into supplementation. However higher doses (2 and 4 g/day) only approached significance, possibly due to small sample sizes in these groups (Peet & Horrobin, 2002). In another study, EPA improved depressive mood relative to placebo beginning 2 weeks into supplementation (Nemets et al., 2002). Lespérance *et al.* (2011) found that 8 weeks of EPA did not significantly reduce depression scores relative to placebo in a heterogeneous sample of individuals with major depressive disorder with and without anxiety disorders. However when analysis was restricted to individuals with major depressive disorder without anxiety disorders, EPA significantly reduced depression scores relative to placebo (Lesperance et al., 2011). A lower dose improved depression scores relative to placebo after 4 weeks of supplementation (Su et al., 2003). da Silva *et al.* (2008) assessed the influence of *n*-3 PUFA in patients with Parkinson's Disease and MDD, who were or were not taking antidepressants, and results indicated reduced depressive symptoms in both *n*-3 PUFA groups (with and without antidepressants) relative to both placebo groups (da Silva et al., 2008). One final study evaluated the influence of *n*-3 PUFA on depressive symptoms in women with psychological distress (PD), with and without major depressive episode (MDE) (Lucas et al., 2009). They found that *n*-3 PUFA improved depressive symptoms relative to placebo in participants with no history of MDE.

Although such mixed findings may suggest that *n*-3 PUFA intake has negligible influence on mood, methodological limitations may have masked beneficial effects. Notably, only two RCTs included lead-in phases to screen for placebo responders (Carney et al., 2009; Su et al., 2003). Su *et al.* (2003) eliminated subjects who experienced a 20% or greater reduction in depressive symptoms during a 1 week lead-in phase and still found a beneficial influence of EPA on depressive scores relative to placebo (Su et al., 2003). Conversely, Carney et al (2009)

eliminated subjects who no longer scored above 16 on the Beck Depression Inventory after a 2 week lead-in phase in which subjects received sertraline and a placebo resembling the fish oil treatment and ultimately found no differences between *n*-3 PUFA supplementation and placebo. Because between 12.5% (Su et al., 2003) and 31% (Carney et al., 2009) were excluded during the lead-in phase, studies that did not control for placebo responders in this way may have failed to fully expose between-group differences.

Additionally, it is important to note the different ratios of EPA to DHA in the studies that found beneficial effects of *n*-3 PUFAs on depressive mood versus those that found no influence, as previous reviews have suggested that EPA has greater antidepressant efficacy than DHA (Martins, 2009; Parker et al., 2006). Support for a beneficial effect of EPA over DHA comes from studies that found associations between *n*-3 PUFA intake and reductions in depressive symptoms which generally employed higher EPA:DHA ratios (i.e. 100% EPA, 2:1 or 1.5:1 EPA:DHA) (da Silva et al., 2008; Lesperance et al., 2011; Nemets et al., 2002; Peet & Horrobin, 2002; Su et al., 2003) than studies that used either DHA alone, or relatively low EPA:DHA ratio, the highest being 1.24:1 EPA:DHA and found no effects (Carney et al., 2009; Grenyer et al., 2007; Marangell et al., 2003; Rogers et al., 2008; Silvers et al., 2005).

However, other evidence calls to question the differential mood-altering effects of EPA and DHA. First, not all RCTs following this pattern: Lucas *et al.* (2009) found that 1.06 EPA + 0.15 DHA g/day reduced depressive scores but only in women without depressive episodes, and Antypa *et al.* (2011) found no difference in depressive symptoms after 1.74 EPA + 0.25 DHA g/day. Second, the majority of studies have used 100% EPA or high EPA:DHA ratios, which may create a selection bias in finding positive effects for these EPA:DHA ratios. Third, to our knowledge only one study to date has specifically compared the antidepressant action of EPA to

DHA in the same RCT. Sinn and colleagues (2011) found that both EPA- and DHA-rich fish oil improved depressive symptoms in older adults with mild cognitive impairment (Sinn et al., 2012). Finally, DHA is the most abundant PUFA in the brain (up to 40% of PUFAs) (Singh, 2005) and DHA may undergo retroconversion to EPA, at rates between 1.4-12% depending on DHA intake (Arterburn, Hall, & Oken, 2006). These findings suggests the fish oil composition chosen in the present study (60% EPA and 40% DHA) falls within the range of similar studies finding mood effects (Fontani, et al. 2005a,b; Kiecolt-Glaser et al., 2011), and does not likely account for the limited mood changes.

Thus the present study supports a number of previous findings across epidemiological studies as well as randomized controlled trials that show limited effects of *n*-3 PUFA supplementation on mood in stressful or non-stressful situations.

n-3 effects on cognition

Likewise, *n*-3 PUFA supplementation had no effects on emotion-related cognitive processing. First, FO and OO groups did not differ in utilization of different emotion regulation strategies, as assessed by the Emotion Regulation Questionnaire (Egloff et al., 2006). Emotion regulation refers to cognitive processes that enable individuals to regulate their own emotions, through both conscious and non-conscious processes, by increasing or decreasing negative or positive emotions (Gross, 1999). Multiple emotion regulation strategies have been characterized, two of which include cognitive change, i.e. re-evaluating the situation or emotion response to the situation, and response modulation, i.e. changing emotional response, often emotion-expressive behavior (Gross & Thompson, 2007).

The present study focused on cognitive reappraisal and expressive suppression. Cognitive reappraisal falls under the cognitive change family, and involves reevaluating emotional stimuli in order to reduce the emotional impact, whereas expressive suppression falls under the family of response modulation, and involves reducing expressive response to emotional stimuli (Gross, 2002). Oschner, Silvers & Buhle (2012) developed a model of the cognitive control of emotion (MCCE) in which they identified the neural systems implicated in the model, the first of which includes the DLPFC and posterior PFC and inferior parietal regions. These regions support attention and working memory, and thus are thought to help attend to and update reappraisal goals. The second system includes the anterior cingulate cortex (ACC), generally involved in conflict monitoring, which could facilitate checking emotion generation against reappraisal goals. The third includes the ventrolateral prefrontal cortex, which may help choose the proper emotion reaction in light of appraisal goals. Emotion regulation involves similar brain regions also influenced by *n*-3 PUFA supplementation, namely the DLPFC (McNamara et al., 2010; Ochsner, Silvers, & Buhle, 2012), suggesting that *n*-3 PUFA intake may alter emotion regulation, particularly cognitive reappraisal. Although reappraisal was utilized more often than suppression during the stressful Trier Social Stress Test, *n*-3 PUFA supplementation did not influence spontaneous emotion regulation following the stressor. Likewise, *n*-3 supplementation had no effect on cognitive reappraisal ability using the Cognitive Reappraisal Task (Jackson et al., 2000). However, lack of group differences on this task could be due to weaknesses in task design that limited analysis to the non-stressful condition, as discussed further in the Limitations section.

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 and sustained attention (Fontani, et al. 2005b). However other studies support our null results finding that *n*-3 PUFA supplementation had no influence cognitive processes on response inhibition, facial expression recognition, and memory (Antypa et al., 2009; Karr et al., 2012).

Unlike mood effects, it is possible that the EPA to DHA ratio may play a role in whether *n*-3 PUFA supplementation influences cognitive performance. For example, DHA-rich fish oil lowered reaction time on the Stroop Task relative to olive oil and EPA-rich fish oil lowered self-reported fatigue during high cognitive demand. Both DHA- and EPA-rich fish oil impaired episodic memory on the Names-to-Faces task, but this task was one of five tasks measuring episodic memory, the other four of which did not generate effects, indicating that the influence of fish oil on episodic memory is not entirely reliable (Jackson, Deary, Reay, Scholey, & Kennedy, 2012).

Near-infrared spectroscopy (NIRS) is a brain imaging method that measures light absorbance to calculate oxy-hemoglobin (oxy-HB) and deoxy-hemoglobin (deoxy-HB), which provides an indirect measure of brain activity, particularly in the frontal cortex. Given that previous research had found increased prefrontal activation following DHA treatment (McNamara et al., 2010), this technique could shed further light on the relationship between *n*-3 PUFA intake and prefrontal-related cognition. DHA- but not EPA-rich FO increased oxy-HB and total-HB in participants performing the Stroop Task as well as on tasks measuring executive function and cognitive flexibility (Peg-and-Ball Task, Wisconsin Card Sort Task) and working memory (3-Back Task) (Jackson, Reay, Scholey, & Kennedy, 2012a). These effects were replicated in a subsequent study that compared two doses DHA-rich FO (1 g/day and 2 g/day) to

OO on a number of cognitive tasks, including those measuring episodic memory, psychomotor performance, executive function and working memory. Both doses increased oxy-HB during all cognitive tasks relative to OO and whereas 2 mg/day FO increased total-HB during all tasks, 1 mg/day increased total-HB only during the Stroop Task and Rapid Visual Information Processing task, which measures sustained attention. Thus two studies have found enhanced response inhibition following FO relatively high in DHA content (Fontani, et al. 2005b; Jackson et al., 2012), and the influence of fish oil on Stroop Task performance is further evidenced by increased oxy- and total-HB across multiple studies and doses (Jackson, Reay, Scholey, & Kennedy, 2012a,b). However, the effects on mood and other cognitive measures are less consistent, and it is premature to conclude that relative EPA and DHA concentration impact FO changes to cognitive processes, especially emotion-related cognitive processing addressed in the present study, as very little research has addressed these types of tasks in general, let alone using multiple EPA to DHA ratios.

n-3 effects 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 that heart rate was elevated during stress, but unaffected by *n*-3 PUFA supplementation. These results are in line with previous findings that stress, including the Trier Social Stress Test increases heart rate (Kirschbaum et al., 1993) but *n*-3 PUFA supplementation does not alter this stress-induced change (Delarue et al., 2003). However it should be noted that heart rate data was limited to approximately half of subjects (i.e. =37; 16 FO, 21 OO), opening the possibility that

the sample size was too low to detect stress-induced changes in heart rate between the FO- and OO-supplemented groups.

Consistent with previous results, increased salivary cortisol and IL-1β (Kirschbaum et al., 1993; Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 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.

Strengths and limitations

The present study adds to the growing literature exploring the psychoactive influences of *n*-3 PUFAs in that it addresses a key issue of treatment compliance. Subjects took their capsules in front of an investigator each weekday during the five week period, and sent text messages after taking their capsules on weekends. Eleven subjects (5 FO, 6 OO) missed one day of supplementation and one subject (OO) missed three days, meaning that compliance was over 99% across all subjects. Although we cannot guarantee subjects' honesty on weekends, we can be certain that they consumed their designated treatment during at least 80% of the study duration, or at least 28 out of the possible 35 days.

A primary limitation to the present study is the lack of erythrocyte fatty acid analysis. As mentioned in the introduction, erythrocyte measures of PUFA levels may provide a more accurate measure of how *n*-3 PUFA supplementation influences mood and cognition than comparing FO- to OO-supplemented groups. Mounting evidence suggests that 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 were associated with improved depressive symptoms (Sinn et al., 2011). This is consistent with recent findings 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 between-group differences (i.e. *n*-3 PUFA versus control supplementation). There is evidence to suggest that individuals with major depressive disorder have lower levels of serum *n*-3 PUFA and higher *n*-6:*n*-3 ratios than healthy controls (Maes et al., 1996; Maes et al., 1999; McNamara et al., 2010). Importantly, DHA but not EPA levels were higher in individuals with MDD than healthy controls (McNamara et al., 2010). Studies have also found positive correlations between the ratio of total *n*-6 to *n*-3 levels and depression symptom severity. Negative correlations were found between EPA and DHA and depression symptoms severity (Adams, Lawson, Sanigorski, & Sinclair, 1996; Edwards, Peet, Shay, & Horrobin, 1998a; Edwards, Peet, Shay, & Horrobin, 1998b). Analysis of postmortem brains showed lower DHA in the orbitofrontal cortex in individuals with MDD than healthy individuals (McNamara et al., 2007). This deficit was greater in females than males with MDD. Together this research suggests that changes in *n*-3 PUFA levels may be of greater predictive value than the presence or absence of *n*-3 PUFA supplementation on mood measures.

A second limitation pertains to the Cognitive Reappraisal Task, in which we failed to match images in the various conditions (i.e. reappraisal versus maintain, stress versus non-stress, IAPS versus GAPES) for normative valence ratings. Analysis of normative valence ratings following data collection revealed that normative rated pleasantness was higher in reappraise than maintain condition and in the non-stressful than stressful TSST condition. These effects were driven by higher ratings in the reappraise than maintain condition in the stressful but not the non-stressful TSST. Thus, had we analyzed the results in the same manner as other tasks, i.e.

with stress 2(stress, non-stress) as a within-subjects factor, any differences between the stress and non-stress or between the reappraisal and maintain conditions could be due to inherent differences in stimuli rather than the manipulation itself. Instead, we limited analysis to the non-stressful condition, and saw that ratings of unpleasantness were lower during reappraisal than maintain trials but there were differences between the FO and OO groups. However, lack of a stressful comparison makes it impossible to determine whether *n*-3 PUFA supplementation influences stress-induced changes to cognitive reappraisal ability.

A third limitation could potentially lie in the sample size of 72 participants. Although our sample size exceeds that of previous studies finding positive results (e.g. Fontani, et al. 2005a,b), the mean diet Group effect size was small (i.e. mean between-subjects $\eta^2 = .003$). Post-hoc power analysis using results from the executive control component of the Trail Making Test (effect size using $\eta^2 = .14$; Karr et al., 2012) and given a criterion alpha of 0.05 and power of 0.95 revealed that the estimated minimum sample size was 154 (with a critical F of 3.03). Thus the current sample size may have been too low to detect significant effects between FO and OO groups.

Other limitations do not necessarily pertain to the study design but rather to how to best 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 grams per week in the present sample. Although the Food and Drug Administration has not specified a recommended daily allowance, the American Heart Association recommends eating two servings of fatty fish per week, in addition to ALA-rich foods such as flaxseed, 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 2.18 g/week EPA+DHA (Kris-Etherton, Harris, Appel, & Nutrition Committee, 2003), meaning that subjects in the present study consumed well over minimum *n*-3 PUFA intake requirements. Evidence suggests a relationship between erythrocyte *n*-3 PUFA levels and depression and cognition. For example, individuals with major depressive disorder had lower erythrocyte DHA levels than healthy controls (McNamara et al., 2010) and older adults with mild cognitive impairment had lower erythrocyte EPA and higher erythrocyte *n*-6 PUFAs (Milte et al., 2011). Higher erythrocyte EPA was associated with better cognitive function in older adults with history of major depressive disorder than healthy controls (Chiu et al., 2012). Such preliminary findings suggest that individuals deficient in *n*-3 PUFAs could benefit more from supplementation than those with adequate levels. Future trials should address this possibility by comparing the mood and cognitive influence of FO to OO supplementation in individuals within initially low *n*-3 PUFA levels.

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. Support for a role of *n*-3 PUFA in stress-induced changes to mood comes from findings that *n*-3 PUFA supplementation may influence depressive symptoms in part through a mechanism involving inflammation (Sinclair et al., 2007). However, despite research showing that acute stressors such as the Trier Social Stress Test indeed increase pro-inflammatory cytokines, more chronic-type stressors may be necessary to induce longer-lasting elevations in proinflammatory cytokines, and thus greater opportunity for *n*-3 PUFAs to exert anti-inflammatory actions (Brydon et al., 2005; Steptoe et al., 2001; Yamakawa et al., 2009). Additionally, the effect sizes in the relationship between depression and inflammation are generally larger in clinic-based relative to community

samples, suggesting a dose-dependent relationship between proinflammatory cytokine productions and depressive symptoms (Howren, Lamkin, & Suls, 2009), thereby suggesting, again, that the present population of healthy individuals exposed to an acute rather than chronic stressor may not be an acceptable target population to evaluate potential benefits of *n*-3 PUFA supplementation on stress-induced changes to mood and cognitive processing.

Third, relatively young adults like those in the present study may not gain as many mood and cognitive benefits from *n*-3 PUFA supplementation as older adults. The age range for most randomized controlled trials assessing the influence of *n*-3 PUFA supplementation on depressive mood generally begins at 18 and extends to 60 or 75 (see Table 3 for all ranges). Given that depression prevalence is generally lower in older than younger adults (Westerhof & Keyes, 2010) and FO improved depressive symptoms in older adults with mild cognitive impairment (Sinn et al., 2012), future trials should limit samples to narrower age ranged in order to further determine target populations for which *n*-3 PUFA supplementation may be more beneficial.

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 certain health benefits. For instance, higher MUFA intake was associated with reduced cognitive decline in older adults (Naqvi et al., 2011; Okereke et al., 2012; Solfrizzi et al., 2006; Vercambre, Grodstein, & Kang, 2010), reduced depressive symptoms in older adults (Kyrozis et al., 2009) and longevity (Solfrizzi et al., 2005). Additional evidence that OO may confer some cognitive benefit comes from studies focusing on the "Mediterranean Diet," which includes olive oil in addition to fruits, vegetables, cereals, dairy products, spices, onions, fish and white meat, as well as wine in moderation (Bach-Faig et al., 2011). The majority of studies assessing the association between adherence to the Mediterranean Diet and cognitive function

found some protective effect of the Mediterranean diet against age-related cognitive decline in healthy older adults (Tangney et al., 2011; Valls-Pedret et al., 2012) as well as those with cognitive impairment or Alzheimer's disease (Feart, Samieri, & Barberger-Gateau, 2010; Roberts et al., 2010; Scarmeas et al., 2009). In young adults, adherence to the Mediterranean Diet improved mood but had few effects on cognitive function (McMillan, Owen, Kras, & Scholey, 2011). Thus comparing FO to OO supplementation may mask beneficial effects of FO relative to a true placebo, containing only physiological inert substances. Although no trials have specifically evaluated the psychoactive effects of MUFA intake, such research would be informative in choosing an appropriate control treatment in *n*-3 PUFA supplementation studies.

Concluding remarks

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. Four cross-sectional studies found an inverse relationship between *n*-3 PUFA intake and depressive symptoms within the general population (Panagiotakos et al., 2010; Suzuki et al., 2004; Tanskanen et al., 2001; Timonen et al., 2004), whereas three did not (Appleton et al., 2007; Jadoon et al., 2012; Murakami et al., 2008). Likewise, two prospective studies found inverse associations between PUFA intake and depressive symptoms (Astorg et al., 2008; Colangelo et al., 2009) whereas three found null or inconsistent results (Hakkarainen et al., 2004; Lucas et al., 2011; Sanchez-Villegas et al., 2007). Randomized controlled trials are similarly mixed, with six studies finding beneficial effects of *n*-3 PUFA supplementation relative to placebo on depressive symptoms in individuals with MDD (da Silva et al., 2008; Lesperance et al., 2011; Lucas et al., 2009; Nemets et al., 2002; Peet & Horrobin, 2002; Su et al., 2003), and

seven studies finding no influence (Carney et al., 2009; Grenyer et al., 2007; Marangell et al., 2003; Rogers et al., 2008; Silvers et al., 2005). Randomized controlled trials in healthy individuals 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), and reduced feelings of fatigue, but not depression (Antypa et al., 2009).

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. Multiple studies have found positive effects of maternal and formula supplementation on infant cognitive development, particularly in problem solving, memory and language development (Drover et al., 2011; Helland, Smith, Saarem, Saugstad, & Drevon, 2003; Henriksen et al., 2008; Lauritzen, Jorgensen, Olsen, Straarup, & Michaelsen, 2005; Meldrum et al., 2012), but others have found no differences, suggesting that although *n*-3 PUFA supplementation may not influence global cognitive development among infants, it may aid particular cognitive functions. The opposite may be true in older adulthood, as prospective and cross-sectional studies suggest that higher *n*-3 PUFA intake and plasma levels are association with reduced overall cognitive decline with less evidence for specific cognitive domains (e.g. Heude, Ducimetiere, Berr, & EVA Study, 2003; Milte et al., 2011; Samieri et al., 2008). Epidemiological results are supported by some RCTs showing that *n*-3 PUFA supplementation, particularly DHA, reverses age-related cognitive decline in otherwise healthy individuals (Johnson et al., 2008; van de Rest et al., 2008; Yurko-Mauro et al., 2010), but there is less evidence to suggest such an effect in individuals with mild cognitive impairment and Alzheimer's disease. In young adults, two studies have found enhanced response inhibition following fish oil relatively high in DHA content (Fontani, et al. 2005b; Jackson et al., 2012),

and the influence of fish oil on Stroop Task performance is further evidenced by increased oxy- and total-HB across multiple studies and doses (Jackson, Reay, Scholey, & Kennedy et al., 2012a,b). However, the effects other cognitive measures are less consistent.

Despite lack of clear evidence *n*-3 PUFA intake improves mood or cognitive processing, multiple methodological limitations in extant literature may mask beneficial effects, including failures to include lead in phases to eliminate placebo responders, measure erythrocyte PUFA levels to quantify individual differences in how changes in *n*-3 PUFA levels predict changes in mood, and use of olive oil as a control treatment which itself my improve some aspects of mood and cognition (e.g. Kyrozis et al., 2009; Solfrizzi et al., 2005; Solfrizzi et al., 2006). 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.

References

Adams, P. B., Lawson, S., Sanigorski, A., & Sinclair, A. J. (1996). Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. *Lipids, 31 Suppl*, S157-61.

Antypa, N., Smelt, A. H., Strengholt, A., & Van der Does, A. W. (2011). Effects of omega-3 fatty acid supplementation on mood and emotional information processing in recovered depressed individuals. *Journal of Psychopharmacology (Oxford, England),* doi:10.1177/0269881111424928

Antypa, N., Van der Does, A. J., Smelt, A. H., & Rogers, R. D. (2009). Omega-3 fatty acids (fish-oil) and depression-related cognition in healthy volunteers. *Journal of Psychopharmacology (Oxford, England), 23*(7), 831-840. doi:10.1177/0269881108092120

Appleton, K. M., Gunnell, D., Peters, T. J., Ness, A. R., Kessler, D., & Rogers, P. J. (2008). No clear evidence of an association between plasma concentrations of n-3 long-chain polyunsaturated fatty acids and depressed mood in a non-clinical population. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 78*(6), 337-342. doi:10.1016/j.plefa.2008.04.009

Appleton, K. M., Peters, T. J., Hayward, R. C., Heatherley, S. V., McNaughton, S. A., Rogers, P. J., . . . Kessler, D. (2007). Depressed mood and n-3 polyunsaturated fatty acid intake from fish: Non-linear or confounded association? *Social Psychiatry and Psychiatric Epidemiology, 42*(2), 100-104. doi:10.1007/s00127-006-0142-3

Arterburn, L. M., Hall, E. B., & Oken, H. (2006). Distribution, interconversion, and dose response of n-3 fatty acids in humans. *The American Journal of Clinical Nutrition, 83*(6 Suppl), 1467S-1476S.

Astorg, P., Couthouis, A., Bertrais, S., Arnault, N., Meneton, P., Guesnet, P., . . . Hercberg, S. (2008). Association of fish and long-chain n-3 polyunsaturated fatty acid intakes with the occurrence of depressive episodes in middle-aged french men and women. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 78*(3), 171-182. doi:10.1016/j.plefa.2008.01.003

Auestad, N., Halter, R., Hall, R. T., Blatter, M., Bogle, M. L., Burks, W., . . . Bornstein, M. H. (2001). Growth and development in term infants fed long-chain polyunsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics, 108*(2), 372-381.

Auestad, N., Scott, D. T., Janowsky, J. S., Jacobsen, C., Carroll, R. E., Montalto, M. B., . . . Hall, R. T. (2003). Visual, cognitive, and language assessments at 39 months: A follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics, 112*(3 Pt 1), e177-83.

Bach-Faig, A., Berry, E. M., Lairon, D., Reguant, J., Trichopoulou, A., Dernini, S., . . . Mediterranean Diet Foundation Expert Group. (2011). Mediterranean diet pyramid today. science and cultural updates. *Public Health Nutrition, 14*(12A), 2274-2284. doi:10.1017/S1368980011002515; 10.1017/S1368980011002515

Benton, D. (2010). Neurodevelopment and neurodegeneration: Are there critical stages for nutritional intervention? *Nutrition Reviews, 68 Suppl 1*, S6-10. doi:10.1111/j.1753-4887.2010.00324.x; 10.1111/j.1753-4887.2010.00324.x

Brunyé, T. T., Howe, J. L., & Mahoney, C. R. (2013). Acute exercise suppresses judgments of facial emotion intensity. *Motivation and Emotion,* doi:DOI 10.1007/s11031-013-9341-x

Brydon, L., Edwards, S., Jia, H., Mohamed-Ali, V., Zachary, I., Martin, J. F., & Steptoe, A. (2005). Psychological stress activates interleukin-1beta gene expression in human mononuclear cells. *Brain, Behavior, and Immunity, 19*(6), 540-546. doi:10.1016/j.bbi.2004.12.003

Calder, P. C. (2006). Polyunsaturated fatty acids and inflammation. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 75*(3), 197-202. doi:10.1016/j.plefa.2006.05.012

Calder, P. C. (2009). Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie, 91*(6), 791-795. doi:10.1016/j.biochi.2009.01.008

Calder, P. C. (2010). Omega-3 fatty acids and inflammatory processes. *Nutrients, 2*(3), 355-374. doi:10.3390/nu2030355

Calder, P. C., Albers, R., Antoine, J. M., Blum, S., Bourdet-Sicard, R., Ferns, G. A., . . . Zhao, J. (2009). Inflammatory disease processes and interactions with nutrition. *The British Journal of Nutrition, 101 Suppl 1*, S1-45. doi:10.1017/S0007114509377867

Carney, R. M., Freedland, K. E., Rubin, E. H., Rich, M. W., Steinmeyer, B. C., & Harris, W. S. (2009). Omega-3 augmentation of sertraline in treatment of depression in patients with

coronary heart disease: A randomized controlled trial. *JAMA : The Journal of the American Medical Association, 302*(15), 1651-1657. doi:10.1001/jama.2009.1487

Chiu, C. C., Frangou, S., Chang, C. J., Chiu, W. C., Liu, H. C., Sun, I. W., . . . Stewart, R. (2012). Associations between n-3 PUFA concentrations and cognitive function after recovery from late-life depression. *The American Journal of Clinical Nutrition, 95*(2), 420-427. doi:10.3945/ajcn.111.015784; 10.3945/ajcn.111.015784

Chiu, C. C., Su, K. P., Cheng, T. C., Liu, H. C., Chang, C. J., Dewey, M. E., . . . Huang, S. Y. (2008). The effects of omega-3 fatty acids monotherapy in alzheimer's disease and mild cognitive impairment: A preliminary randomized double-blind placebo-controlled study. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 32*(6), 1538-1544. doi:10.1016/j.pnpbp.2008.05.015; 10.1016/j.pnpbp.2008.05.015

Colangelo, L. A., He, K., Whooley, M. A., Daviglus, M. L., & Liu, K. (2009). Higher dietary intake of long-chain omega-3 polyunsaturated fatty acids is inversely associated with depressive symptoms in women. *Nutrition (Burbank, Los Angeles County, Calif.), 25*(10), 1011-1019. doi:10.1016/j.nut.2008.12.008

da Silva, T. M., Munhoz, R. P., Alvarez, C., Naliwaiko, K., Kiss, A., Andreatini, R., & Ferraz, A. C. (2008). Depression in parkinson's disease: A double-blind, randomized, placebo-controlled pilot study of omega-3 fatty-acid supplementation. *Journal of Affective Disorders, 111*(2-3), 351-359. doi:10.1016/j.jad.2008.03.008

Dacks, P. A., Shineman, D. W., & Fillit, H. M. (2013). Current evidence for the clinical use of long-chain polyunsaturated n-3 fatty acids to prevent age-related cognitive decline and

alzheimer's disease. *The Journal of Nutrition, Health & Aging, 17*(3), 240-251. doi:10.1007/s12603-012-0431-3; 10.1007/s12603-012-0431-3

Dan-Glauser, E. S., & Scherer, K. R. (2011). The geneva affective picture database (GAPED): A new 730-picture database focusing on valence and normative significance. *Behavior Research Methods, 43*(2), 468-477. doi:10.3758/s13428-011-0064-1; 10.3758/s13428-011-0064-1

Delarue, J., Matzinger, O., Binnert, C., Schneiter, P., Chiolero, R., & Tappy, L. (2003). Fish oil prevents the adrenal activation elicited by mental stress in healthy men. *Diabetes & Metabolism, 29*(3), 289-295.

Djousse, L., Akinkuolie, A. O., Wu, J. H., Ding, E. L., & Gaziano, J. M. (2012). Fish consumption, omega-3 fatty acids and risk of heart failure: A meta-analysis. *Clinical Nutrition (Edinburgh, Scotland), 31*(6), 846-853. doi:10.1016/j.clnu.2012.05.010; 10.1016/j.clnu.2012.05.010

Dolcos, F., & McCarthy, G. (2006). Brain systems mediating cognitive interference by emotional distraction. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 26*(7), 2072-2079. doi:10.1523/JNEUROSCI.5042-05.2006

Drover, J. R., Hoffman, D. R., Castaneda, Y. S., Morale, S. E., Garfield, S., Wheaton, D. H., & Birch, E. E. (2011). Cognitive function in 18-month-old term infants of the DIAMOND study: A randomized, controlled clinical trial with multiple dietary levels of docosahexaenoic acid. *Early Human Development, 87*(3), 223-230. doi:10.1016/j.earlhumdev.2010.12.047; 10.1016/j.earlhumdev.2010.12.047

Edwards, R., Peet, M., Shay, J., & Horrobin, D. (1998a). Depletion of docosahexaenoic acid in red blood cell membranes of depressive patients. *Biochemical Society Transactions, 26*(2), S142.

Edwards, R., Peet, M., Shay, J., & Horrobin, D. (1998b). Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *Journal of Affective Disorders, 48*(2-3), 149-155.

Egloff, B., Schmukle, S. C., Burns, L. R., & Schwerdtfeger, A. (2006). Spontaneous emotion regulation during evaluated speaking tasks: Associations with negative affect, anxiety expression, memory, and physiological responding. *Emotion (Washington, D.C.), 6*(3), 356-366. doi:10.1037/1528-3542.6.3.356

Erk, S., Kleczar, A., & Walter, H. (2007). Valence-specific regulation effects in a working memory task with emotional context. *NeuroImage, 37*(2), 623-632. doi:10.1016/j.neuroimage.2007.05.006

Feart, C., Samieri, C., & Barberger-Gateau, P. (2010). Mediterranean diet and cognitive function in older adults. *Current Opinion in Clinical Nutrition and Metabolic Care, 13*(1), 14-18. doi:10.1097/MCO.0b013e3283331fe4; 10.1097/MCO.0b013e3283331fe4

Ferraz, A. C., Delattre, A. M., Almendra, R. G., Sonagli, M., Borges, C., Araujo, P., . . . Lima, M. M. (2011). Chronic omega-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol. *Behavioural Brain Research, 219*(1), 116-122. doi:10.1016/j.bbr.2010.12.028

Fontani, G., Corradeschi, F., Felici, A., Alfatti, F., Bugarini, R., Fiaschi, A. I., . . . Berra, B. (2005). Blood profiles, body fat and mood state in healthy subjects on different diets supplemented with omega-3 polyunsaturated fatty acids. *European Journal of Clinical Investigation, 35*(8), 499-507. doi:10.1111/j.1365-2362.2005.01540.x

Fontani, G., Corradeschi, F., Felici, A., Alfatti, F., Migliorini, S., & Lodi, L. (2005). Cognitive and physiological effects of omega-3 polyunsaturated fatty acid supplementation in healthy subjects. *European Journal of Clinical Investigation, 35*(11), 691-699. doi:10.1111/j.1365-2362.2005.01570.x

Freund-Levi, Y., Basun, H., Cederholm, T., Faxen-Irving, G., Garlind, A., Grut, M., . . . Eriksdotter-Jonhagen, M. (2008). Omega-3 supplementation in mild to moderate alzheimer's disease: Effects on neuropsychiatric symptoms. *International Journal of Geriatric Psychiatry, 23*(2), 161-169. doi:10.1002/gps.1857

Freund-Levi, Y., Eriksdotter-Jonhagen, M., Cederholm, T., Basun, H., Faxen-Irving, G., Garlind, A., . . . Palmblad, J. (2006). Omega-3 fatty acid treatment in 174 patients with mild to moderate alzheimer disease: OmegAD study: A randomized double-blind trial. *Archives of Neurology, 63*(10), 1402-1408. doi:10.1001/archneur.63.10.1402

Gerra, G., Zaimovic, A., Mascetti, G. G., Gardini, S., Zambelli, U., Timpano, M., . . . Brambilla, F. (2001). Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. *Psychoneuroendocrinology, 26*(1), 91-107.

Giles, G. E., Mahoney, C. R., & Kanarek, R. B. (2013). Omega-3 fatty acids influence mood in healthy and depressed individuals.*In press.*

Grenyer, B. F., Crowe, T., Meyer, B., Owen, A. J., Grigonis-Deane, E. M., Caputi, P., & Howe, P. R. (2007). Fish oil supplementation in the treatment of major depression: A randomised double-blind placebo-controlled trial. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 31*(7), 1393-1396. doi:10.1016/j.pnpbp.2007.06.004

Gros, D. F., Antony, M. M., Simms, L. J., & McCabe, R. E. (2007). Psychometric properties of the state-trait inventory for cognitive and somatic anxiety (STICSA): Comparison to the state-trait anxiety inventory (STAI). *Psychological Assessment, 19*(4), 369-381. doi:10.1037/1040-3590.19.4.369

Gross, J. J. (1999). Emotion regulation: Past, present, future. *Cognition and Emotion, 13*(5), 551-551-773.

Gross, J. J., & Thompson, R. A. (2007). Emotion regulation conceptual foundations. In J. J. Gross (Ed.), *Handbook of emotion regulation* [null] (pp. 3-3-24). New York, NY: Guilford Publications.

Gross, J. J. (2002). Emotion regulation: Affective, cognitive, and social consequences. *Psychophysiology, 39*(3), 281-291. doi:10.1017.S0048577201393198

Hakkarainen, R., Partonen, T., Haukka, J., Virtamo, J., Albanes, D., & Lonnqvist, J. (2004). Is low dietary intake of omega-3 fatty acids associated with depression? *The American Journal of Psychiatry, 161*(3), 567-569.

Helland, I. B., Smith, L., Saarem, K., Saugstad, O. D., & Drevon, C. A. (2003). Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics, 111*(1), e39-44.

Hellhammer, J., & Schubert, M. (2012). The physiological response to trier social stress test relates to subjective measures of stress during but not before or after the test. *Psychoneuroendocrinology, 37*(1), 119-124. doi:10.1016/j.psyneuen.2011.05.012

Henriksen, C., Haugholt, K., Lindgren, M., Aurvag, A. K., Ronnestad, A., Gronn, M., . . . Drevon, C. A. (2008). Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. *Pediatrics, 121*(6), 1137-1145. doi:10.1542/peds.2007-1511; 10.1542/peds.2007-1511

Heude, B., Ducimetiere, P., Berr, C., & EVA Study. (2003). Cognitive decline and fatty acid composition of erythrocyte membranes--the EVA study. *The American Journal of Clinical Nutrition, 77*(4), 803-808.

Hooper, L., Thompson, R. L., Harrison, R. A., Summerbell, C. D., Ness, A. R., Moore, H. J., . . . Davey Smith, G. (2006). Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: Systematic review. *BMJ (Clinical Research Ed.), 332*(7544), 752-760. doi:10.1136/bmj.38755.366331.2F

Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: A meta-analysis. *Psychosomatic Medicine, 71*(2), 171-186. doi:10.1097/PSY.0b013e3181907c1b

Jackson, D. C., Malmstadt, J. R., Larson, C. L., & Davidson, R. J. (2000). Suppression and enhancement of emotional responses to unpleasant pictures. *Psychophysiology, 37*(4), 515-522.

Jackson, P. A., Deary, M. E., Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2012). No effect of 12 weeks' supplementation with 1 g DHA-rich or EPA-rich fish oil on cognitive function or mood in healthy young adults aged 18-35 years. *The British Journal of Nutrition, 107*(8), 1232-1243. doi:10.1017/S000711451100403X; 10.1017/S000711451100403X

Jackson, P. A., Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2012a). DHA-rich oil modulates the cerebral haemodynamic response to cognitive tasks in healthy young adults: A near IR spectroscopy pilot study. *The British Journal of Nutrition, 107*(8), 1093-1098. doi:10.1017/S0007114511004041; 10.1017/S0007114511004041

Jackson, P. A., Reay, J. L., Scholey, A. B., & Kennedy, D. O. (2012b). Docosahexaenoic acid-rich fish oil modulates the cerebral hemodynamic response to cognitive tasks in healthy young adults. *Biological Psychology, 89*(1), 183-190. doi:10.1016/j.biopsycho.2011.10.006; 10.1016/j.biopsycho.2011.10.006

Jadoon, A., Chiu, C. C., McDermott, L., Cunningham, P., Frangou, S., Chang, C. J., . . . Stewart, R. (2012). Associations of polyunsaturated fatty acids with residual depression or anxiety in older people with major depression. *Journal of Affective Disorders, 136*(3), 918-925. doi:10.1016/j.jad.2011.09.007

Johnson, E. J., McDonald, K., Caldarella, S. M., Chung, H. Y., Troen, A. M., & Snodderly, D. M. (2008). Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein

supplementation in older women. *Nutritional Neuroscience, 11*(2), 75-83. doi:10.1179/147683008X301450; 10.1179/147683008X301450

Joormann, J., & Gotlib, I. H. (2006). Is this happiness I see? biases in the identification of emotional facial expressions in depression and social phobia. *Journal of Abnormal Psychology, 115*(4), 705-714. doi:10.1037/0021-843X.115.4.705

Karr, J. E., Grindstaff, T. R., & Alexander, J. E. (2012). Omega-3 polyunsaturated fatty acids and cognition in a college-aged population. *Experimental and Clinical Psychopharmacology, 20*(3), 236-242. doi:10.1037/a0026945; 10.1037/a0026945

Kessler, R. C., Zhao, S., Blazer, D. G., & Swartz, M. (1997). Prevalence, correlates, and course of minor depression and major depression in the national comorbidity survey. *Journal of Affective Disorders, 45*(1-2), 19-30.

Kiecolt-Glaser, J. K. (2010). Stress, food, and inflammation: Psychoneuroimmunology and nutrition at the cutting edge. *Psychosomatic Medicine, 72*(4), 365-369. doi:10.1097/PSY.0b013e3181dbf489

Kiecolt-Glaser, J. K., Belury, M. A., Andridge, R., Malarkey, W. B., & Glaser, R. (2011). Omega-3 supplementation lowers inflammation and anxiety in medical students: A randomized controlled trial. *Brain, Behavior, and Immunity, 25*(8), 1725-1734. doi:10.1016/j.bbi.2011.07.229

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'trier social stress test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*(1-2), 76-81.

Koenigs, M., & Grafman, J. (2009). The functional neuroanatomy of depression: Distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behavioural Brain Research, 201*(2), 239-243. doi:10.1016/j.bbr.2009.03.004

Kotwal, S., Jun, M., Sullivan, D., Perkovic, V., & Neal, B. (2012). Omega 3 fatty acids and cardiovascular outcomes: Systematic review and meta-analysis. *Circulation.Cardiovascular Quality and Outcomes, 5*(6), 808-818. doi:10.1161/CIRCOUTCOMES.112.966168; 10.1161/CIRCOUTCOMES.112.966168

Kris-Etherton, P. M., Harris, W. S., Appel, L. J., & Nutrition Committee. (2003). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(2), e20-30.

Kudielka, B. M., Schommer, N. C., Hellhammer, D. H., & Kirschbaum, C. (2004). Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. *Psychoneuroendocrinology, 29*(8), 983-992. doi:10.1016/j.psyneuen.2003.08.009

Kuhlmann, S., Piel, M., & Wolf, O. T. (2005). Impaired memory retrieval after psychosocial stress in healthy young men. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 25*(11), 2977-2982. doi:10.1523/JNEUROSCI.5139-04.2005

Kyrozis, A., Psaltopoulou, T., Stathopoulos, P., Trichopoulos, D., Vassilopoulos, D., & Trichopoulou, A. (2009). Dietary lipids and geriatric depression scale score among elders: The EPIC-greece cohort. *Journal of Psychiatric Research, 43*(8), 763-769. doi:10.1016/j.jpsychires.2008.09.003; 10.1016/j.jpsychires.2008.09.003

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2005). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual.technical report A-6.* . Gainesville, FL: University of Florida.

Lauritzen, L., Jorgensen, M. H., Olsen, S. F., Straarup, E. M., & Michaelsen, K. F. (2005). Maternal fish oil supplementation in lactation: Effect on developmental outcome in breast-fed infants. *Reproduction, Nutrition, Development, 45*(5), 535-547. doi:10.1051/rnd:2005044

Lesperance, F., Frasure-Smith, N., St-Andre, E., Turecki, G., Lesperance, P., & Wisniewski, S. R. (2011). The efficacy of omega-3 supplementation for major depression: A randomized controlled trial. *The Journal of Clinical Psychiatry, 72*(8), 1054-1062. doi:10.4088/JCP.10m05966blu

Lieberman, H. R., Mays, M. Z., Shukitt-Hale, B., Chinn, K. S. K., & Tharion, W. J. (1996). Effects of sleeping in a chemical protective mask on sleep quality and performance. *Aviation, Space, and Environmental Medicine, 67*(9), 841–848.

Lin, P. Y., Huang, S. Y., & Su, K. P. (2010). A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. *Biological Psychiatry, 68*(2), 140-147. doi:10.1016/j.biopsych.2010.03.018

Lucas, M., Asselin, G., Merette, C., Poulin, M. J., & Dodin, S. (2009). Ethyl-eicosapentaenoic acid for the treatment of psychological distress and depressive symptoms in middle-aged women: A double-blind, placebo-controlled, randomized clinical trial. *The American Journal of Clinical Nutrition, 89*(2), 641-651. doi:10.3945/ajcn.2008.26749

Lucas, M., Mirzaei, F., O'Reilly, E. J., Pan, A., Willett, W. C., Kawachi, I., . . . Ascherio, A. (2011). Dietary intake of n-3 and n-6 fatty acids and the risk of clinical depression in women: A 10-y prospective follow-up study. *The American Journal of Clinical Nutrition, 93*(6), 1337-1343. doi:10.3945/ajcn.111.011817

Luchtman, D. W., & Song, C. (2013). Cognitive enhancement by omega-3 fatty acids from child-hood to old age: Findings from animal and clinical studies. *Neuropharmacology, 64*, 550-565. doi:10.1016/j.neuropharm.2012.07.019; 10.1016/j.neuropharm.2012.07.019

Maes, M., Christophe, A., Delanghe, J., Altamura, C., Neels, H., & Meltzer, H. Y. (1999). Lowered omega3 polyunsaturated fatty acids in serum phospholipids and cholesteryl esters of depressed patients. *Psychiatry Research, 85*(3), 275-291.

Maes, M., Smith, R., Christophe, A., Cosyns, P., Desnyder, R., & Meltzer, H. (1996). Fatty acid composition in major depression: Decreased omega 3 fractions in cholesteryl esters and increased C20: 4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. *Journal of Affective Disorders, 38*(1), 35-46.

Marangell, L. B., Martinez, J. M., Zboyan, H. A., Kertz, B., Kim, H. F., & Puryear, L. J. (2003). A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. *The American Journal of Psychiatry, 160*(5), 996-998.

Martins, J. G. (2009). EPA but not DHA appears to be responsible for the efficacy of omega-3 long chain polyunsaturated fatty acid supplementation in depression: Evidence from a meta-analysis of randomized controlled trials. *Journal of the American College of Nutrition, 28*(5), 525-542.

McMillan, L., Owen, L., Kras, M., & Scholey, A. (2011). Behavioural effects of a 10-day mediterranean diet. results from a pilot study evaluating mood and cognitive performance. *Appetite, 56*(1), 143-147. doi:10.1016/j.appet.2010.11.149; 10.1016/j.appet.2010.11.149

McNair, D. M., Lorr, M., & Droppleman, L. F. (1971). *Profile of mood states*. San Diego, CA: Educational and Industrial Testing Service.

McNamara, R. K., Able, J., Jandacek, R., Rider, T., Tso, P., Eliassen, J. C., . . . Adler, C. M. (2010). Docosahexaenoic acid supplementation increases prefrontal cortex activation during sustained attention in healthy boys: A placebo-controlled, dose-ranging, functional magnetic resonance imaging study. *The American Journal of Clinical Nutrition, 91*(4), 1060-1067. doi:10.3945/ajcn.2009.28549

McNamara, R. K., Hahn, C. G., Jandacek, R., Rider, T., Tso, P., Stanford, K. E., & Richtand, N. M. (2007). Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. *Biological Psychiatry, 62*(1), 17-24. doi:10.1016/j.biopsych.2006.08.026

McNamara, R. K., Jandacek, R., Rider, T., Tso, P., Dwivedi, Y., & Pandey, G. N. (2010). Selective deficits in erythrocyte docosahexaenoic acid composition in adult patients with

bipolar disorder and major depressive disorder. *Journal of Affective Disorders, 126*(1-2), 303-311. doi:10.1016/j.jad.2010.03.015

Meldrum, S. J., D'Vaz, N., Simmer, K., Dunstan, J. A., Hird, K., & Prescott, S. L. (2012). Effects of high-dose fish oil supplementation during early infancy on neurodevelopment and language: A randomised controlled trial. *The British Journal of Nutrition, 108*(8), 1443-1454. doi:10.1017/S0007114511006878; 10.1017/S0007114511006878

Meyer, B. J., Grenyer, B. F., Crowe, T., Owen, A. J., Grigonis-Deane, E. M., & Howe, P. R. (2013). Improvement of major depression is associated with increased erythrocyte DHA. *Lipids,* doi:10.1007/s11745-013-3801-7

Milte, C. M., Sinn, N., Street, S. J., Buckley, J. D., Coates, A. M., & Howe, P. R. (2011). Erythrocyte polyunsaturated fatty acid status, memory, cognition and mood in older adults with mild cognitive impairment and healthy controls. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 84*(5-6), 153-161. doi:10.1016/j.plefa.2011.02.002; 10.1016/j.plefa.2011.02.002

Murakami, K., Mizoue, T., Sasaki, S., Ohta, M., Sato, M., Matsushita, Y., & Mishima, N. (2008). Dietary intake of folate, other B vitamins, and omega-3 polyunsaturated fatty acids in relation to depressive symptoms in japanese adults. *Nutrition (Burbank, Los Angeles County, Calif.), 24*(2), 140-147. doi:10.1016/j.nut.2007.10.013

Naqvi, A. Z., Harty, B., Mukamal, K. J., Stoddard, A. M., Vitolins, M., & Dunn, J. E. (2011). Monounsaturated, trans, and saturated fatty acids and cognitive decline in women. *Journal of*

the American Geriatrics Society, 59(5), 837-843. doi:10.1111/j.1532-5415.2011.03402.x; 10.1111/j.1532-5415.2011.03402.x

Nemets, B., Stahl, Z., & Belmaker, R. H. (2002). Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. *The American Journal of Psychiatry, 159*(3), 477-479.

Nobmann, E. D., Ponce, R., Mattil, C., Devereux, R., Dyke, B., Ebbesson, S. O., . . . GOCADAN study, 2. (2005). Dietary intakes vary with age among eskimo adults of northwest alaska in the GOCADAN study, 2000-2003. *The Journal of Nutrition, 135*(4), 856-862.

O'Brien, S. M., Scott, L. V., & Dinan, T. G. (2004). Cytokines: Abnormalities in major depression and implications for pharmacological treatment. *Human Psychopharmacology, 19*(6), 397-403. doi:10.1002/hup.609

Ochsner, K. N., Bunge, S. A., Gross, J. J., & Gabrieli, J. D. (2002). Rethinking feelings: An FMRI study of the cognitive regulation of emotion. *Journal of Cognitive Neuroscience, 14*(8), 1215-1229. doi:10.1162/089892902760807212

Ochsner, K. N., Silvers, J. A., & Buhle, J. T. (2012). Functional imaging studies of emotion regulation: A synthetic review and evolving model of the cognitive control of emotion. *Annals of the New York Academy of Sciences, 1251*, E1-24. doi:10.1111/j.1749-6632.2012.06751.x; 10.1111/j.1749-6632.2012.06751.x

O'Connor, D. L., Hall, R., Adamkin, D., Auestad, N., Castillo, M., Connor, W. E., . . . Ross Preterm Lipid Study. (2001). Growth and development in preterm infants fed long-chain

polyunsaturated fatty acids: A prospective, randomized controlled trial. *Pediatrics, 108*(2), 359-371.

Okereke, O. I., Rosner, B. A., Kim, D. H., Kang, J. H., Cook, N. R., Manson, J. E., . . . Grodstein, F. (2012). Dietary fat types and 4-year cognitive change in community-dwelling older women. *Annals of Neurology, 72*(1), 124-134. doi:10.1002/ana.23593; 10.1002/ana.23593

Panagiotakos, D. B., Mamplekou, E., Pitsavos, C., Kalogeropoulos, N., Kastorini, C. M., Papageorgiou, C., . . . Stefanadis, C. (2010). Fatty acids intake and depressive symptomatology in a greek sample: An epidemiological analysis. *Journal of the American College of Nutrition, 29*(6), 586-594.

Parker, G., Gibson, N. A., Brotchie, H., Heruc, G., Rees, A. M., & Hadzi-Pavlovic, D. (2006). Omega-3 fatty acids and mood disorders. *The American Journal of Psychiatry, 163*(6), 969-978. doi:10.1176/appi.ajp.163.6.969

Peet, M., & Horrobin, D. F. (2002). A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Archives of General Psychiatry, 59*(10), 913-919.

Quinn, J. F., Raman, R., Thomas, R. G., Yurko-Mauro, K., Nelson, E. B., Van Dyck, C., . . . Aisen, P. S. (2010). Docosahexaenoic acid supplementation and cognitive decline in alzheimer disease: A randomized trial. *JAMA : The Journal of the American Medical Association, 304*(17), 1903-1911. doi:10.1001/jama.2010.1510; 10.1001/jama.2010.1510

Raedler, T. J. (2011). Inflammatory mechanisms in major depressive disorder. *Current Opinion in Psychiatry, 24*(6), 519-525. doi:10.1097/YCO.0b013e32834b9db6

Ree, M. J., French, D., MacLeod, C., & Locke, V. (2008). Distinguishing cognitive and somatic dimensions of state and trait anxiety: Development and validation of the state-trait inventory for cognitive and somatic anxiety (STICSA). *Behavioural and Cognitive Psychotherapy, 36*(3), 313-313-332.

Roberts, R. O., Geda, Y. E., Cerhan, J. R., Knopman, D. S., Cha, R. H., Christianson, T. J., . . . Petersen, R. C. (2010). Vegetables, unsaturated fats, moderate alcohol intake, and mild cognitive impairment. *Dementia and Geriatric Cognitive Disorders, 29*(5), 413-423. doi:10.1159/000305099; 10.1159/000305099

Rogers, P. J., Appleton, K. M., Kessler, D., Peters, T. J., Gunnell, D., Hayward, R. C., . . . Ness, A. R. (2008). No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: A randomised controlled trial. *The British Journal of Nutrition, 99*(2), 421-431. doi:10.1017/S0007114507801097

Samieri, C., Feart, C., Letenneur, L., Dartigues, J. F., Peres, K., Auriacombe, S., . . . Barberger-Gateau, P. (2008). Low plasma eicosapentaenoic acid and depressive symptomatology are independent predictors of dementia risk. *The American Journal of Clinical Nutrition, 88*(3), 714-721.

Sanchez-Villegas, A., Henriquez, P., Figueiras, A., Ortuno, F., Lahortiga, F., & Martinez-Gonzalez, M. A. (2007). Long chain omega-3 fatty acids intake, fish consumption and mental

disorders in the SUN cohort study. *European Journal of Nutrition, 46*(6), 337-346. doi:10.1007/s00394-007-0671-x

Scarmeas, N., Stern, Y., Mayeux, R., Manly, J. J., Schupf, N., & Luchsinger, J. A. (2009). Mediterranean diet and mild cognitive impairment. *Archives of Neurology, 66*(2), 216-225. doi:10.1001/archneurol.2008.536; 10.1001/archneurol.2008.536

Schoofs, D., Preuss, D., & Wolf, O. T. (2008). Psychosocial stress induces working memory impairments in an n-back paradigm. *Psychoneuroendocrinology, 33*(5), 643-653. doi:10.1016/j.psyneuen.2008.02.004

Scott, D. T., Janowsky, J. S., Carroll, R. E., Taylor, J. A., Auestad, N., & Montalto, M. B. (1998). Formula supplementation with long-chain polyunsaturated fatty acids: Are there developmental benefits? *Pediatrics, 102*(5), E59.

Silvers, K. M., Woolley, C. C., Hamilton, F. C., Watts, P. M., & Watson, R. A. (2005). Randomised double-blind placebo-controlled trial of fish oil in the treatment of depression. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 72*(3), 211-218. doi:10.1016/j.plefa.2004.11.004

Simopoulos, A. P. (2011). Importance of the omega-6/omega-3 balance in health and disease: Evolutionary aspects of diet. *World Review of Nutrition and Dietetics, 102*, 10-21. doi:10.1159/000327785

Sinclair, A. J., Begg, D., Mathai, M., & Weisinger, R. S. (2007). Omega 3 fatty acids and the brain: Review of studies in depression. *Asia Pacific Journal of Clinical Nutrition, 16 Suppl 1*, 391-397.

Singh, M. (2005). Essential fatty acids, DHA and human brain. *Indian Journal of Pediatrics, 72*(3), 239-242.

Sinn, N., Milte, C. M., Street, S. J., Buckley, J. D., Coates, A. M., Petkov, J., & Howe, P. R. (2011). Effects of n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: A 6-month randomised controlled trial. *The British Journal of Nutrition,* , 1-12. doi:10.1017/S0007114511004788

Sinn, N., Milte, C. M., Street, S. J., Buckley, J. D., Coates, A. M., Petkov, J., & Howe, P. R. (2012). Effects of n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: A 6-month randomised controlled trial. *The British Journal of Nutrition, 107*(11), 1682-1693. doi:10.1017/S0007114511004788; 10.1017/S0007114511004788

Solfrizzi, V., Colacicco, A. M., D'Introno, A., Capurso, C., Torres, F., Rizzo, C., . . . Panza, F. (2006). Dietary intake of unsaturated fatty acids and age-related cognitive decline: A 8.5-year follow-up of the italian longitudinal study on aging. *Neurobiology of Aging, 27*(11), 1694-1704. doi:10.1016/j.neurobiolaging.2005.09.026

Solfrizzi, V., D'Introno, A., Colacicco, A. M., Capurso, C., Palasciano, R., Capurso, S., . . . Panza, F. (2005). Unsaturated fatty acids intake and all-causes mortality: A 8.5-year follow-

up of the italian longitudinal study on aging. *Experimental Gerontology, 40*(4), 335-343. doi:10.1016/j.exger.2005.01.003

Steptoe, A., Willemsen, G., Owen, N., Flower, L., & Mohamed-Ali, V. (2001). Acute mental stress elicits delayed increases in circulating inflammatory cytokine levels. *Clinical Science (London, England : 1979), 101*(2), 185-192.

Stonehouse, W., Conlon, C. A., Podd, J., Hill, S. R., Minihane, A. M., Haskell, C., & Kennedy, D. (2013). DHA supplementation improved both memory and reaction time in healthy young adults: A randomized controlled trial. *The American Journal of Clinical Nutrition, 97*(5), 1134-1143. doi:10.3945/ajcn.112.053371; 10.3945/ajcn.112.053371

Stough, C., Downey, L., Silber, B., Lloyd, J., Kure, C., Wesnes, K., & Camfield, D. (2012). The effects of 90-day supplementation with the omega-3 essential fatty acid docosahexaenoic acid (DHA) on cognitive function and visual acuity in a healthy aging population. *Neurobiology of Aging, 33*(4), 824.e1-824.e3. doi:10.1016/j.neurobiolaging.2011.03.019; 10.1016/j.neurobiolaging.2011.03.019

Su, H. M. (2010). Mechanisms of n-3 fatty acid-mediated development and maintenance of learning memory performance. *The Journal of Nutritional Biochemistry, 21*(5), 364-373. doi:10.1016/j.jnutbio.2009.11.003

Su, K. P., Huang, S. Y., Chiu, C. C., & Shen, W. W. (2003). Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology, 13*(4), 267-271.

Sugano, M. (1996). Characteristics of fats in japanese diets and current recommendations. *Lipids, 31 Suppl*, S283-6.

Sun, Q., Ma, J., Campos, H., Hankinson, S. E., & Hu, F. B. (2007). Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *The American Journal of Clinical Nutrition, 86*(1), 74-81.

Suzuki, S., Akechi, T., Kobayashi, M., Taniguchi, K., Goto, K., Sasaki, S., . . . Uchitomi, Y. (2004). Daily omega-3 fatty acid intake and depression in japanese patients with newly diagnosed lung cancer. *British Journal of Cancer, 90*(4), 787-793. doi:10.1038/sj.bjc.6601621

Tangney, C. C., Kwasny, M. J., Li, H., Wilson, R. S., Evans, D. A., & Morris, M. C. (2011). Adherence to a mediterranean-type dietary pattern and cognitive decline in a community population. *The American Journal of Clinical Nutrition, 93*(3), 601-607. doi:10.3945/ajcn.110.007369; 10.3945/ajcn.110.007369

Tanskanen, A., Hibbeln, J. R., Tuomilehto, J., Uutela, A., Haukkala, A., Viinamaki, H., . . . Vartiainen, E. (2001). Fish consumption and depressive symptoms in the general population in finland. *Psychiatric Services (Washington, D.C.), 52*(4), 529-531.

Timonen, M., Horrobin, D., Jokelainen, J., Laitinen, J., Herva, A., & Rasanen, P. (2004). Fish consumption and depression: The northern finland 1966 birth cohort study. *Journal of Affective Disorders, 82*(3), 447-452. doi:10.1016/j.jad.2004.02.002

Trofimiuk, E., & Braszko, J. J. (2011). Long-term administration of cod liver oil ameliorates cognitive impairment induced by chronic stress in rats. *Lipids, 46*(5), 417-423. doi:10.1007/s11745-011-3551-3

Tufts University School of Medicine, Nutrition Infection Unit. (2002). HIV nutrition and health: Omega-3 fatty acids. Retrieved 07/29, 2013, from http://www.tufts.edu/tufts-test/med/nutrition-HIV/hiv/health_omega3.htm

Urry, H. L. (2009). Using reappraisal to regulate unpleasant emotional episodes: Goals and timing matter. *Emotion (Washington, D.C.), 9*(6), 782-797. doi:10.1037/a0017109

Valls-Pedret, C., Lamuela-Raventos, R. M., Medina-Remon, A., Quintana, M., Corella, D., Pinto, X., . . . Ros, E. (2012). Polyphenol-rich foods in the mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. *Journal of Alzheimer's Disease : JAD, 29*(4), 773-782. doi:10.3233/JAD-2012-111799; 10.3233/JAD-2012-111799

van de Rest, O., Geleijnse, J. M., Kok, F. J., van Staveren, W. A., Dullemeijer, C., Olderikkert, M. G., . . . de Groot, C. P. (2008). Effect of fish oil on cognitive performance in older subjects: A randomized, controlled trial. *Neurology, 71*(6), 430-438. doi:10.1212/01.wnl.0000324268.45138.86; 10.1212/01.wnl.0000324268.45138.86

Vercambre, M. N., Grodstein, F., & Kang, J. H. (2010). Dietary fat intake in relation to cognitive change in high-risk women with cardiovascular disease or vascular factors. *European Journal of Clinical Nutrition, 64*(10), 1134-1140. doi:10.1038/ejcn.2010.113; 10.1038/ejcn.2010.113

Westerhof, G. J., & Keyes, C. L. (2010). Mental illness and mental health: The two continua model across the lifespan. *Journal of Adult Development, 17*(2), 110-119. doi:10.1007/s10804-009-9082-y

What does a serving size look like? (2007). Retrieved 10/25, 2011, from http://www.fitsugar.com/What-Does-Serving-Size-Look-Like-762806

Wolf, R. C., & Walter, H. (2005). Evaluation of a novel event-related parametric fMRI paradigm investigating prefrontal function. *Psychiatry Research, 140*(1), 73-83. doi:10.1016/j.pscychresns.2005.06.002

Yamakawa, K., Matsunaga, M., Isowa, T., Kimura, K., Kasugai, K., Yoneda, M., . . . Ohira, H. (2009). Transient responses of inflammatory cytokines in acute stress. *Biological Psychology, 82*(1), 25-32. doi:10.1016/j.biopsycho.2009.05.001; 10.1016/j.biopsycho.2009.05.001

Young, A. W., Rowland, D., Calder, A. J., Etcoff, N. L., Seth, A., & Perrett, D. I. (1997). Facial expression megamix: Tests of dimensional and category accounts of emotion recognition. *Cognition, 63*(3), 271-313.

Yurko-Mauro, K., McCarthy, D., Rom, D., Nelson, E. B., Ryan, A. S., Blackwell, A., . . . MIDAS Investigators. (2010). Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association, 6*(6), 456-464. doi:10.1016/j.jalz.2010.01.013; 10.1016/j.jalz.2010.01.013

TABLES

Table 1 Cross-sectional studies assessing the association between n-3 PUFA intake and depressive symptoms

Authors	Geographic Region	Sample n (% female)	Mood Measures	Results
Tanskanen et al., 2001	Finland	3,204 (NR)	Beck Depression Inventory (BDI)	Inverse association between fish intake and depressive symptoms in females only
Timonen et al., 2004	Finland	5,689 (52%)	Hopkins Symptom Checklist-25 (HSLC-25), MDD diagnosis	Inverse association between frequency of fish intake and depression and suicide in females only
Suzuki et al., 2004	Japan	771 (28%)[a]	Hospital Anxiety and Depression Scale (HAD)	Inverse association between ALA and total *n*-3 PUFA intake; no associations between total EPA or DHA intake and depressive symptoms
Appleton et al., 2007	UK	2,982 (66%)	Depression, Anxiety and Stress Scales (DASS-21)	Inverse association between *n*-3 PUFA intake and depressive symptoms; no associations after controlling for age, social deprivation and *n*-3 PUFA supplement use
Murakami et al., 2008	Japan	517 (40%)	Center for Epidemiological Studies Depression Scale (CES-D)	No associations between PUFA intake and depressive symptoms
Panagiotakos et al., 2010	Greece	453 (88%)	Zung Depression Rating Scale (ZDRS)	Inverse association between PUFA (total *n*-3, DHA, EPA, ALA, LA) and mono-unsaturated FA (MUFA) intake and depressive symptoms; positive relationship between saturated FA (SFA) intake and depressive symptoms
Jadoon et al., 2012	Taiwan	135 (72.7%)[b]	Hamilton Depression Rating Scale (HDRS)	No associations between PUFA intake and depressive symptoms

[a] Cancer patients
[b] 68±6.6 years
NR not reported

Table 2 Prospective studies assessing the association between n-3 PUFA intake and depressive symptoms

Authors	Geographic Region	Sample n (% female)	Follow-up Duration	Mood Measures	Results
Hakkarainen et al., 2004	Finland	29,133 (0%)	5-8 years	Self-reported depressed mood, MDD treatment, suicide	Trends for increased depression risk in highest and lowest tertile of fish consumption
Sanchez-Villegas et al., 2007	Spain	7,903 (NR%)	2 years	Depression, Anxiety, Stress diagnoses	Inverse association between moderate fish intake and risk of mental disorder in females only, but not after controlling for *n*-3 PUFA intake
Astorg et al., 2008	France	1,864 (57%)	2 years	Depressive episode occurrence	Inverse association between moderate to high fish intake and risk of depressive episode; stronger association in males than females and in non-smokers than smokers
Colangelo et al., 2009	USA	3,317 (55%)	10 years	CES-D	Inverse association between EPA, DHA, EPA+DHA, and fish intake and depressive symptoms in females only; similar results after excluding individuals taking antidepressants
Lucas et al., 2011	USA	54,632 (100%)	10 years	Clinical depression diagnosis	ALA, EPA+DHA, AA intake not associated with risk of clinical depression; positive associations between increased LA intake and risk of clinical depression; negative associations between ALA:AA and *n*-3:*n*-6 ratios and risk of depression

NR not reported

Table 3 Clinical Trials assessing the influence of n-3 PUFA on depressive symptoms in individuals with MDD or MDE.

Authors	Sample n (% female)	Age range	Mean age±SD *n-3*, placebo	Diagnosis	Concurrent pharmacological/ behavioral treatment	*n*-3 PUFA Manipulation	Mood Measures	Duration (weeks)	Results
Peet & Horrobin, 2002	70 (84%)	18-70	48 (1 g), 43 (2 g), 44 (4 g), 44 (placebo)	Depression	Tricyclic antidepressant (n=14), SSRIs (n-50), other antidepressant (n=6)	1, 2, or 4 g/day ethyl-eicosapentaenoate (E-EPA)	HDRS, BDI, Montgomery-Asberg Depression Rating(MADRS)	12	Depression scores decreased in 1 g/day dose only
Nemets et al., 2002	20 (85%)	18-75	54.2±13.9, 52.1±10.2	MDD	Antidepressants	2 g/day E-EPA	HDRS	4	E-EPA reduced depression scores relative to placebo
Su et al., 2003	22 (82%)	18-60	35.2±11.6, 42.3±10.7	MDD	Antidepressants fluvoxamine, trazodone, moclobemide; oral sedatives/hypnotics permitted	1.32 g *n*-3 PUFA (.88 g EPA, .44 g DHA)	HDRS	8	E-EPA reduced depression scores relative to placebo
Marangell et al., 2003	35 (80%)	18-65	46.8±11.6, 49.9±11.2	MDD	None	2 g/day DHA	HDRS, MADRS, Global Assessment of Functioning Scale (GAF)	6	No differences in depression scores between DHA and placebo groups
Silvers et al., 2005	77 (53%)	18-65	39.8±11.9, 37.7±13.6	MDE	Antidepressants (n=61); psychotherapy (n=21)	3 g/day *n*-3 PUFA (0.6 g EPA, 2.4 g DHA)	HDRS, BDI	12	No differences in depression scores between DHA and placebo groups
Grenyer et al., 2007	83 (61%)	18-70	45.27 [c]	MDD	Tricyclic antidepressant (n=4), SSRIs (n-33), other antidepressant (n=22)	3 g/day fish oil (0.6 g EPA, 2.2 g DHA)	HDRS, BDI, GAF	16	No differences in depression scores between fish oil and placebo groups
da Silva et al., 2008	31 (58%)	49-48	64.4 [c]	MDD + Parkinson's Disease (PD)	Antidepressants > 1 year (n=16)	1.2 g/day *n*-3 PUFA (.72 g EPA, .48 g DHA)	MADRS, BDI, Clinical Global Impression Scale (CGI)	12	Depression scores decreased in *n*-3 PUFA group, with and without concurrent antidepressants
Rogers et al., 2008	120 (77%)	18-70	38.8±13, 38.2±13.7	Mild to moderate depression	None	1.5 g/day *n*-3 PUFA (.63 g EPA, .85 g DHA)	DASS, BDI, State-Trait Anger Expression Inventory (STAXI)	12	No differences in depression scores between *n*-3 PUFA and placebo groups
Carney et al., 2009	122 (34%)	NR	58.1±9.4, 58.6±8.5	MDD + Coronary Heart Disease (CDH)	None	50 mg/day sertraline + 2 g/day *n*-3 PUFA (.93 g EPA, .75 g DHA)[b]	BDI, HDRS	10	No differences in depression scores between *n*-3 PUFA and placebo groups
Lucas et al., 2009	120 (100%)	40-55	48.4±4.0, 49.1±3.9	Psychological distress (PD) with or without MDE	None	1.2 g/day *n*-3 PUFA (1.05 g EPA, 0.15 g DHA)	HDRS, CGI, HSLC, Psychological General Well-Being (PGWB)	8	*n*-3 PUFA reduced depression scores relative to placebo in women without MDE only
Lesperance et al., 2011	432 (65.8%)	≥18	46.6±11.54, 45.4±13.27	MDE + Anxiety Disorder[c]	Antidepressants (n=174), psychotherapy (n=64), other psychotropic medications (n=117)	1.65 g/day *n*-3 PUFA (1.5 g EPA, .15 g DHA)	MADRS, Inventory of Depressive Symptomatology ($IDS\text{-}SR_{30}$)	8	Non-significant reduction in depression scores in EPA group relative to placebo. Reduction significant in individuals without anxiety disorders.
Antypa et al., 2011	106 (82%)	18-65	25.8±11.8, 23.5±6.0	Recovered MDD	Antidepressants (n=7)	2.3 g/day fish oil (1.74 g EPA, .25 g DHA)	BDI, Leiden Index of Depression-Sensitivity-Revised (LEIDS-R)	4	Self-reported depression and tension decreased in fish oil relative to placebo group, no differences in depressive symptoms between *n*-3 PUFA and placebo groups
Meyer et al., 2013	83 (X)	18-75	X	MDD	Counseling	8 g/day tuna oil (.6 g EPA, 2 g DHA)	BDI, HDRS	16	No differences in depression scores between *n*-3 PUFA and placebo groups

NR Not reported
a Mean age for all subjects combined
b 50 mg/day sertraline (SSRI) in both *n*-3 PUFA and placebo groups
c n=228 with comorbid anxiety disorder

Table 4 Placebo-controlled trials assessing the influence of n-3 PUFA on mood in healthy individuals

Authors	Sample n (% female)	Age range	Mean age±SD, *n-3*, placebo	*n*-3 PUFA Manipulation	Duration (weeks)	Mood Measures	Results
Fontani et al., 2005a	33 (60%)	22-51	33±7[a]	4 g/day fish oil (1.6 g EPA, 0.8 g DHA)	5	POMS (Profile of Mood States)	Increase in vigor, decrease in anger, anxiety, fatigue, depression, and confusion in fish oil relative to placebo group
Fontani et al., 2005b	36 (89%)	22-51	33±3[a]	4 g/day fish oil (1.6 g EPA, 0.8 g DHA)	5	POMS	Increase in vigor, decrease in anger, anxiety, fatigue, depression, and confusion in fish oil relative to placebo group
Antypa et al., 2009	54 (NR)	18-27	22.6±3.6, 22.6±4.1	3 g/day fish oil (1.74 g EPA, 0.25 g DHA)	4	Mini International Neuropsychiatric Interview (M.I.N.I.), BDI-II, POMS, Behavioral Inhibition/Behavioral Activation Scales (BIS/BAS), LEIDS-R,	Decrease in fatigue, control/perfectionism, and risk aversion in fish oil relative to placebo group
Kiecolt-Glaser et al., 2011	68 (44%)	21-29	23.9±2.02, 23.4±1.7	2.496 g/day *n*-3 PUFA (.2085 g EPA, .388 g DHA)	6 or 12	CES-D, Beck Anxiety Inventory (BAI)	Decrease in anxiety but not depression in *n*-3 PUFA relative to placebo group

[a] Average for all subjects
NR not reported

Table 5 Randomized controlled trials assessing the influence of infant n-3 PUFA supplementation on cognitive development

Authors	Sample n (female) term	Gestational age	*n*-3 PUFA Manipulation	Supplement Duration (months)	Age at testing (months)	Cognitive Measures	Results
Scott et al., 1998	274 (NR) term	NR	0.2% DHA formula versus 0.12% DHA + 0.43% AA formula versus control formula versus BF[a]	14	12, 14	BSID[b] MCDI[c]	No differences BSID DHA < BF vocabulary comprehension DHA < control vocabulary production
Auestad et al., 2001	404 (203) term	*Egg-DTG*[d]*:* 39.0±1.3, *FO*[e]*-Fungal:* 39.3±1.2, *Control:* 39.4±1.2, *Egg-DTG BF:* 39.6±1.3, *Control BF:* 39.2±1.2	FO + fungal oil formula (.46 g/100 g AA, ≤0.04 g/100 g EPA, 0.13 g/100 g DHA) versus Egg-DTG formula (0.45 g/100 g AA, 0.14 g/100 g DHA) versus Control formula versus BF + Egg-DTG versus BF control	12	1, 2, 4, 6, 9, 12, 14	BSID MCDI FTII[f] Infant Behavior Questionnaire	No differences BSID, FTII FO-Fungal > Egg-DTG Formula vocabulary expression Egg-DTG Formula < Control Formula smiling and laughter (Infant Behavior Questionnaire)
O'Connor et al., 2001	470 (214) preterm	*Egg-DTG:* 29.7±2.0, *FO-Fungal:* 29.8±2.1, *Control:* 29.6±1.9, *Breastfed:* 29.7±2.1	FO + fungal oil formula (.43 g/100 g AA, .08 g/100 g EPA, .27 g/100 g DHA) versus Egg-DTG formula (.41 g/100 g AA, .24 g/100 g DHA) versus Control formula versus BF	12	2, 4, 6, 9, 14	BSID MCDI FTII	No differences BSID, except FO-Fungal > Control motor score in infants ≤1250 g birth weight Egg-DTG > Fish-Fungal, Control groups novelty preference No differences MCDI, except Egg-DTG, FO-Fungal < Control vocabulary comprehension after removing infants from Spanish-speaking families
Auestad et al., 2003	157 (71) term	*All:* 39±1	ARA+DHA (.43 g/100g ARA + .12 g/100g DHA) versus DHA (.23 g/100 g DHA) versus control formula versus BF	52	14, 39	SBIS[g] PPVT-R[h] BVM[i] MLU[j]	No differences
Henriksen et al., 2008	141 (64) preterm	*n-3:* 28.4± NR *Control:* 28.9± NR	48 mg/kg day DHA + 48 mg/kg day AA versus soy oil + medium-chain triglyceride oil	2.25 (median)	6	Ages and Stages Questionnaire Recognition memory via ERP[k]	*n*-3 > Control problem solving *n*-3 > Control recognition memory
Drover et al., 2011	131 (52) term	*All:* 37-42	0.32% DHA (17 mg/100 kcal), 0.64% DHA (34 mg/100kcal) or 0.96%DHA (54 mg/100 kcal) + 0.64% ARA (54 mg/100kcal) versus Control formula	12	18	BSID BRS[l]	No differences between groups analyzed separately Combined DHA groups > Control mental development, language Combined DHA groups > Controls BRS emotion regulation
Meldrum et al., 2012	420 (139) term	*FO:* 39.1±1.1, *Control:* 29.4±1.3	FO (230 mg DHA, 110 mg EPA) versus OO[m]	6	12, 18	BSID MCDI CBCL[n]	FO > Control MCDI later developing gestures, and total number gestures No differences BSID FO > Control CBCL Anxious/depressed behaviors

[a] Breastfed (BF)
[b] Bayley Scales of Infant Development (BSID)
[c] MacArthur Communicative Development Inventories (MCDI)
[d] Egg-derived triglyceride (Egg-DTG)
[e] Fish oil (FO)
[f] Fagan Test of Infant Intelligence (FTII)
[g] Stanford-Binet Intelligence Scale (SBIS) Form L-M
[h] Peabody Picture Vocabulary Test-Revised (PPVT-R)
[i] Beery Visual-Motor Index Test (BVM)
[j] Mean length of utterance (MLU)
[k] Event Related Potential (ERP)
[l] Behavior Rating Scale (BRS)
[m] Olive oil (OO)
[n] Achenbach Child Behavior Checklist (CBCL)
NR Not reported

Table 6 Randomized Controlled Trials assessing the influence of n-3 PUFA supplementation on cognitive function in healthy older adults

Authors	Sample n (female)	Age range mean±SD)	*n*-3 PUFA Manipulation	Duration (weeks)	Cognitive Measures	Results
Johnson et al., 2008	49 (49)	60-80 *DHA:* 68.5±1.3, *Lutein:* 66.7±1.9, *DHA+Lutein:* 68.6±1.3, *Control:* 68.0±1.2	800 mg/day DHA versus 12 mg/day Lutein versus DHA+Lutein versus placebo	16	Verbal Fluency, Digit Span Forward and Backward, Shopping Test Task, Word List Memory Test, Memory in Reality Apartment Test, Stroop Test, NES2 Mood Scales: self-reported mood	DHA, Lutein, DHA+Lutein > Control verbal fluency DHA + Lutein < Control response time on Shopping List Memory Test, delayed recall in Memory in Reality Apartment Test
van de Rest et al., 2008	196 (88)	> 65 *High EPA-DHA:* 69.9±3.4, *Low EPA-DHA:* 69.53.2. *Control:* 70.1±3.7	900 mg/day fish oil high EPA-DHA (226±3 mg EPA, 176±4 mg DHA) versus low EPA-DHA (2093±17 mg EPA, 847±23 mg DHA) versus sunflower oil	26	Verbal Fluency, Word Learning Test, Digit Span Forward and Backward, Trail Making Test version A and B, Stroop Test	Low EPA-DHA < Control memory at 13 but not 26 weeks Low and High EPA-DHA> Control attention at 26 weeks in *APOE ε4* carriers only Low EPA-DHA > Control attention at 26 weeks in men only
Yurko-Mauro et al., 2010	485 (282)	≥ 55 *DHA:* 70±9.3, *Control:* 70±8.7	Algal triglyceride oil (900 mg/day DHA) versus corn+soy oil	24	WAIS-III[a] logical memory, MMSE[b], CANTAB[c] Subtests: PAL[d], PRM[e], VRM[f], SOC[g], SWM[h], Frequency of Forgetting-10 Scale, ADCS-ADL PI[i], Geriatric Depression Scale[j]	DHA > Control CANTAB PAL, VRM
Stough et al., 2012	74 (43)	45-77 *DHA:* 55.08±8.70 *Control:* 57.66±8.67	1000 mg/day tuna oil (252 mg DHA, 60 mg EPA) versus soybean oil	90 days	STAI[k], Cognitive Drug Research (CDR)[l]	No differences CDR factors

[a] Wechsler Memory Scale (III) (WAIS-III)
[b] Mini Mental State Exam (MMSE)
[c] Cambridge Neuropsychological Test Automated Battery (CANTAB)
[d] Paired Associative Learning (PAL)
[e] Pattern Recognition Memory (PRM)
[f] Verbal Recognition Memory (VRM)
[g] Stockings of Cambridge (SOC)
[h] Spatial Working Memory (SWM)
[i] Alzheimer's Disease Cooperative Study-Activities of Daily Living Prevention Instrument (ADCS-ADL PI)
[j] Geriatric Depression Scale (GDS)
[k] State Trait Anxiety Inventory (STAI)
[l] Cognitive Drug Research (CDR) assessment

Table 7 Randomized Controlled Trials assessing the influence on n-3 PUFA supplementation on cognitive function in older adults with mild cognitive impairment or Alzheimer's disease

Authors	Sample n (female)	Age range (mean±SD)	*n*-3 PUFA Manipulation	Duration (weeks)	Cognitive Measures	Results
Freund-Levi et al., 2006	204 (110) AD[a]	*n*-3 PUFA: 72.6±9.0, Placebo: 72.9±8.6	*n*-3 PUFA (150 mg EPA, 430 mg DHA) versus corn oil	24 (+ 24 weeks open label *n*-3 for all participants)	MMSE[b], ADAS-cog[c], CDR[d]	No differences MMSE or ADAS-cog across all participants *n*-3 > Control MMSE delayed recall, attention (very mild AD) *n*-3 > Control ADAS-cog delayed recall (severe AD)
Chiu et al., 2008	43 (20) mild-moderate AD (23) or MCI[e] (23)	*n*-3 PUFA: 70.1-77.8 (74.0), Placebo: 71.8-81.1 (76.5)	*n*-3 PUFA (1080 mg EPA, 720 mg DHA) versus olive oil	24	ADAS-cog, CIBIC[f], HDRS[g]	*n*-3 > Control CIBIC No differences ADAS-cog, MMSE, HDRS
Freund-Levi et al., 2008	204 (90) AD	*n*-3 PUFA: 72.6±9.0, Placebo: 72.9±8.6	*n*-3 PUFA (600 mg EPA, 1700 mg DHA) versus corn oil	24 (+ 24 weeks open label *n*-3 for all participants)	NPI[h], MADRS[i], CGB[j], DAD[k]	
Quinn et al., 2010	402 (210) mild-moderate AD	*n*-3 PUFA: 76±9.3, Placebo: 76±7.9	2 g Algal DHA (.9-1.1 g DHA) versus corn or soy oil	72	MMSE (baseline only), ADAS-cog, CDR, ADCS-ADL[l], Quality of Life of Alzheimer's Disease Scale	No differences
Sinn et al., 2012	50 (16) MCI	≥ 65 EPA-rich FO:74.88±5.06 DHA-rich FO: 74.22±7 Control: 73±3.96	EPA-rich fish oil (1.67 g EPA, 0.16 g DHA) versus DHA-rich fish oil (1.55 g DHA, 0.4 g EPA) versus safflower oil (2.2 g LA)	24	GDS[m], SF-36 Health Survey: health and quality of life, RAVLT[n]WAIS-III[o] (Digits Forward, Boston Naming Task, Letter-Number Sequencing, Digits Backward), Trail-Making Task, Stroop Test, Verbal Fluency	EPA, DHA > LA depressive symptoms DHA > LA verbal fluency No differences quality of life

[a] Alzheimer's diease (AD)
[b] Mini Mental State Exam (MMSE)
[c] Cognitive portion of Alzheimer's Disease Assessment Scale (ADAS-cog)
[d] Clinical Dementia Rating (CDR) Scale
[e] Mild cognitive impairment (MCI)
[f] Clinician's Interview-Based Impression of Change (CIBIC) Scale
[g] Hamilton Depression Rating Scale (HDRS)
[h] Neuropsychiatric Inventory (NPI)
[i] Montgomery Asperg Depression Rating Scale (MADRS)
[j] Caregiver Burden Scale (CGB)
[k] Disability Assessment for Dementia Scale (DAD)
[l] ADCS Activities of Daily Living (ADCS-ADL) Scale
[m] Geriatric Depression Scale (GDS)
[n] Rey Auditory Verbal Learning Test (RAVLT)
[o] Wechsler Memory Scale (III) (WAIS-III)

Table 8 Randomized controlled trials assessing the influence of n-3 PUFA supplementation in young adults on cognitive performance

Authors	Sample n (female)	Age range	Mean age±SD	*n*-3 PUFA Manipulation	Duration (weeks)	Cognitive Measures	Results
Fontani et al., 2005a	33 (20)	22-51	33±7	Diet (40% carb, 30% pro, 30% fat versus 55% carb, 15% pro, 30% fat) x FO[a] (4 g/day FO: 0.8 g DHA, 1.6 g EPA versus OO[b])	5	POMS[c]	FO > Control vigor FO < Control anger, anxiety, fatigue, depression, confusion
Fontani et al., 2005b	36 (32)	22-51	FO: 33±7, Control: 33±3	4 g/day FO (0.8 g DHA, 1.6 g EPA) versus OO	5	POMS, SRT[d], CRT[e], GNG[f], Complex GNG (sustained attention)	FO > Control vigor FO < Control anger, anxiety, fatigue, depression, confusion FO < Control GNG reaction time , number of errors No differences SRT, CRT
Antypa et al., 2009	54 (44)	18-27	FO: 22.2±3.6, Control : 22.6±4.1	3 g/day FO (0.25 g DHA, 1.74 g EPA)	4	Affective GNG, Attentional GNG; Facial Expression Recognition Task, Decision-Making (Gambling) Task, M.I.N.I.[g]; BDI-II[h]; POMS; BIS/BAS[i]; LEIDS-R[j]	FO < Control fatigue, LEIDS-R Control/Perfectionism, Risk Aversion and total score FO > Control Gambling Task risk-seeking decision-making in gains only trials No differences GNG, Facial Expression Recognition
Jackson et al., 2012	140 (94)	18-35	DHA-rich FO: 21.96±.54, EPA-rich FO: 22.74±.61, Control: 21.94±.50	1 g/day DHA-rich FO (.45 g DHA, .09 g EPA) versus EPA-rich FO (.2 g DHA, .3 g EPA) versus OO	12	COMPASS[k] CDB[l] Bond-Lader VAS[m] DASS[n]	DHA-rich FO < Control Stroop reaction time EPA-, DHA-rich FO < Control Names-to-Faces Task (episodic memory) EPA-rich FO < Control CBD self-reported fatigue No differences mood , other cognitive measures
Jackson et al. 2012a	22 (13)	X	All: 21.96±X	1 g/day DHA-rich FO (.45 g DHA, .09 g EPA) versus EPA-rich FO (.2 g DHA, .3 g EPA) versus OO	12	Stroop Task Peg-and-Ball Task 3-Back Task Wisconsin Card Sort Task NIRS[o]	DHA-rich FO > Control oxy-HB following DHA-rich FO during Stroop Task DHA-rich FO > Control total-HB during Stroop Task, Peg-and-Ball, 3-Back Tasks
Jackson et al., 2012b	65 (49)	18-29	1 g FO: 20.5±.43, 2 g FO: 19.95±.34. Control : 21.35±.62	1 g/day FO (.45 g DHA, .09 g EPA) versus 2 g/day FO (.9 g DHA, .18 g EPA) versus OO	12	COMPASS	1, 2 g FO > Control oxy-HB during all tasks 2 g FO > Control total-HB during all tasks 1 g FO > FO Control during Stroop, RVIP[p] tasks
Karr et al., 2012	41 (29)	X	FO: 19.90±18.3, Control: 20.43±1.63	1 g/day FO (240 mg DHA, 360 mg EPA) versus coconut oil	4	RAVLT[q], Stroop Test, TMT[r], PANAS[s]	FO > Control final stages (6 and 7) RAVLT FO < Control TMT No differences in Stroop Test, PANAS
Stonehouse et al., 2013	176 (110)	18-45	DHA: 33.4±7.76, Control: 33.2±7.9	1.16 g/day DHA versus sunflower oil	24	COMPASS Kit of Factor Referenced Cognitive Tests (Finding As Task) WAIS-III[t] (Letter-Number Sequencing)	DHA > Control episodic memory in females only DHA > Control working memory in males only No differences attention, processing speed

[a] Fish oil (FO)
[b] Olive oil (OO)
[c] Profile of Mood States (POMS)
[d] Simple Reaction Time (SRT)
[e] Choice Reaction Time (CRT)
[f] Go/No-Go (GNG)
[g] Mini International Neuropsychiatric Interview (M.I.N.I)
[h] Beck Depression Inventory-II (BDI-II)
[i] Behavioral Inhibition/Behavioral Activation Scales (BIS/BAS)
[j] Leiden Index of Depression Sensitivity – Revised (LEIDS-R)
[k] Computerized Mental Performance Assessment System (COMPASS): episodic memory, psychomotor performance, attention, executive function, working memory
[l] Cognitive Demand Battery (CDB)
[m] Visual Analogue Scales (VAS)
[n] Depression, Anxiety, and Stress Scales (DASS)
[o] Near IR spectroscopy (NIRS)
[p] Rapid Visual Information Processing (RVIP)
[q] Rey Auditory Verbal Learning Test (RAVLT)
[r] Trail Making Test (TMT), Parts A and B
[s] Positive and Negative Affect Schedule (PANAS)
[t] Wechsler Adult Intelligence Scale III Intelligence Test (WAIS-III)

Table 9 Between-group (fish oil versus olive oil) differences for all mood, physiological and cognitive measures.

		FO		OO			
		M	SD	M	SD	$\alpha_{critical}$	*p*
POMS	Tension	2.68	4.13	1.21	3.69	.007	.120
	Depression	2.92	0.74	3.49	0.75		.853
	Anger	2.61	0.54	3.11	0.55		.514
	Vigor	8.28	1.07	9.76	1.09		.337
	Fatigue	9.40	0.91	7.76	0.93		.210
	Confusion	3.21	0.59	1.37	0.59		.031
	Total Mood Disturbance	12.92	3.11	7.19	3.15		.199
STICSA-S	Somatic anxiety	14.89	0.55	13.92	0.55	.025	.213
	Cognitive anxiety	16.57	0.76	13.92	0.76		.016*
Heart rate	(bpm)	74.00	3.15	75.16	2.75	.05	.782
Cortisol	(ug/dL)	0.35	0.04	0.42	0.04	.05	.341
IL-1β	pg/mL)	212.36	37.61	220.04	37.61	.05	.337
EIT Accuracy	Negative	0.87	0.04	0.84	0.04	.017	.596
	Neutral	0.77	0.03	0.79	0.03		.685
	Scrambled	0.80	0.03	0.76	0.27		.276
EIT Reaction Time	Negative	1452.66	51.20	1457.41	51.20	.017	.948
	Neutral	1482.18	53.27	1488.90	53.27		.929
	Scrambled	1407.031	59.95	1431.13	59.95		.777
Morphed Faces Intensity Rating	Happy	3.10	0.24	3.15	0.23	.008	.880
	Anger	5.13	0.15	5.14	0.15		.943
	Sad	2.91	0.15	2.61	0.14		.144
	Surprise	3.12	0.22	3.03	0.22		.781
	Fear	4.47	0.14	4.34	0.14		.484
	Disgust	5.26	1.59	3.02	1.59		.323
Morphed Faces Stop Frames	Happy	13.84	2.04	17.05	2.04	.008	.270
	Anger	68.34	2.71	69.42	2.71		.778
	Sad	90.04	1.83	89.40	1.83		.805
	Surprise	19.44	2.29	21.95	2.29		.440
	Fear	78.40	2.80	77.59	2.80		.839
	Disgust	11.78	1.99	13.70	1.99		.498

*No single comparison reached full ($\alpha_{critical}$=.050/# subscales) or partial significance ($\alpha_{critical}$=.025/# subscales), with the exception of the state cognitive anxiety subscale of the STICSA, in which rated cognitive anxiety was higher in the FO than OO group.

Table 10 Means ± standard deviations or number of participants (n) in each group. *p* values denote significance using univariate analysis of variance (ANOVA) for continuous variables.

		FO		OO		
		Mean	SD	Mean	SD	*p*
n (female)		36 (23)		36 (22)		
Age (years)		20.80	2.386	20.49	1.704	0.528
BMI (kg/m^2)		20.45	.73	21.67	.73	0.243
Exercise (hours per week)		3.35	.39	2.76	.39	0.296
n-3 intake (g/week)	Pre-Supplementation	14.62	12.73	12.22	13.37	.445
	Week 1	11.86	10.45	11.13	13.61	.805
	Week 2	10.41	10.10	9.02	10.04	.568
	Week 3	10.35	9.49	11.16	12.59	.767
	Week 4	9.93	10.88	9.60	9.07	.894
	Week 5	8.44	8.30	8.12	7.54	.874
n-6 intake (g/week)	Pre-Supplementation	45.23	44.81	31.60	31.40	.143
	Week 1	36.64	33.26	29.73	26.28	.341
	Week 2	35.49	29.22	24.33	22.07	.149
	Week 3	34.86	42.07	27.97	23.49	.408
	Week 4	32.58	43.58	26.36	27.16	.484
	Week 5	35.79	42.61	25.63	24.10	.253
STICSA-T	Somatic	1.45	0.06	1.34	0.06	0.145
	Cognitive	1.74	0.10	1.69	0.10	0.735

No differences in age, body mass index (BMI), habitual exercise, weekly *n*-3 or *n*-6 PUFA intake or trait anxiety between fish oil (FO) and olive oil (OO) groups pre-supplementation.

Table 11 Profile of Mood States (POMS) change scores (Post – Pre Trier Social Stress Test) means (SEM) for each treatment combination (n=71; 36 FO, 35 OO)

	FO				OO			
	Stress		Non-Stress		Stress		Non-Stress	
	M	SEM	M	SEM	M	SEM	M	SEM
Tension	3.41	0.95	-0.89	0.61	-1.11	0.63	-1.11	0.63
Depression	-0.38	0.83	-1.622	0.65	1.20	0.85	-.94	0.66
Anger	0.70	1.04	-1.378	0.60	3.37	1.07	-.40	0.60
Vigor	-0.68	0.80	-0.24	.727	-.51	0.75	.57	0.82
Fatigue	-1.76	0.65	-1.70	.607	-.74	0.66	-1.66	0.62
Confusion	1.32	0.66	0.08	.433	2.63	0.68	-1.20	0.45
Total Mood Disturbance	3.97	3.34	-5.32	2.37	9.77	3.43	-4.80	2.44

Stress increased rated tension ($p<.001$), depression ($p<.05$), anger ($p<.01$), confusion ($p<.001$), and total mood disturbance ($p<.001$). Rated anger remained stable in the fish oil (FO) group and increased in the OO group ($p<.05$) and rated confusion remained stable in the FO group across both stress and no-stress conditions but increased in the OO group during the stress condition ($p<.05$; see Figure 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).

Table 12 State Trait Inventory of Cognitive and Somatic Anxiety (STICSA) change scores (Post – Pre Trier Social Stress Test) means (SEM) for each treatment combination (n=72; 36 FO, 36 OO)

	FO				OO			
	Stress		Non-Stress		Stress		Non-Stress	
	M	SEM	M	SEM	M	SEM	M	SEM
Somatic	0.86	0.41	-1.64	0.38	0.47	0.41	0.08	0.38
Cognitive	0.72	0.69	-1.00	0.36	0.19	0.69	-0.56	0.36

Stress increased rated somatic ($p<.001$) and cognitive ($p<.05$) anxiety. Rated somatic anxiety was lower following the non-stressful than stressful Trier Social Stress Test in the fish oil (FO) but not olive oil (OO) group ($p<.01$; see Figure 3). No other main effect effects or interaction were found (All ps>.11).

Table 13 Emotion Regulation Questionnaire means (SEM) for each treatment combination (n=72; 36 FO; 36 OO).

	FO				OO			
	Stress		Non-Stress		Stress		Non-Stress	
	M	SEM	M	SEM	M	SEM	M	SEM
Reappraisal	9.75	0.52	9.89	0.53	9.19	0.52	8.47	0.53
Suppression	7.72	0.54	9.58	0.52	7.47	0.54	10.03	0.52

Reappraisal was used more often than suppression during the stressful ($p<.001$) but not non-stressful Trier Social Stress Test ($p>.19$; see Figure 4). No other main effects or interactions were found (all ps$>.13$).

Table 14 Heart rate means in beats per minute (SEM) for each treatment combination (n=37; 16 FO, 21 OO)

	FO				OO			
	Stress		Non-Stress		Stress		Non-Stress	
	M	SEM	M	SEM	M	SEM	M	SEM
Pre-TSST	75.93	2.45	77.00	3.21	76.58	2.14	75.59	2.80
TSST	81.26	2.51	75.64	3.23	81.45	2.19	75.38	2.82
Post-TSST	71.55	2.24	70.92	3.28	72.60	1.95	69.81	2.87

Heart rate was higher than baseline during the TSST and lower than baseline post-TSST ($p<.001$). A Time x Stress interaction ($p<.001$) showed that heart rate was higher during the stressful than control ($p=<.01$) but not before ($p>.98$) or after ($p>.35$) the TSST (see Figure 5). No effects were found for Group, Time x Group, Stress, Stress x Group or Stress x Time x Group (all *ps*>.14).

Table 15 Salivary cortisol means in μg/dL (SEM) for each treatment combination (n=71; 36 FO, 35 OO)

	FO				OO			
	Stress		Non-Stress		Stress		Non-Stress	
	M	SEM	M	SEM	M	SEM	M	SEM
Pre-TSST	0.30	0.06	0.29	0.04	0.49	0.06	0.42	0.04
10 min post-TSST	0.39	0.05	0.31	0.03	0.53	0.05	0.38	0.03
60 min post-TSST	0.24	0.04	0.24	0.03	0.32	0.04	0.25	0.03

Although ANOVAs were performed on log-transformed data, the table shows raw data. Salivary cortisol was higher during the stressful than non-stressful test session ($p<.01$) and higher 10-minutes after the TSST than before or 60-minutes after the TSST ($p<.001$). No differences were found for Group ($p>.10$), Stress x Time ($p>.10$), Stress x Group ($p>.49$), Time x Group ($p>.07$), or Stress x Time x Group ($p>.85$).

Table 15 Salivary interleukin-1β (IL-1β) in pg/mL means (SEM) for each treatment combination (n=72; 36 FO, 36 OO).

	FO		OO	
	M	SEM	M	SEM
Stress	305.15	105.96	149.40	104.96
Non-Stress	178.01	34.08	203.91	34.08

Although ANOVAs were performed on log-transformed data, the table shows raw data. Salivary IL-Iβ was higher during the stressful than non-stressful test session ($p<.05$). No effects were found for Group ($p>.96$) or Stress x Group ($p>.19$).

Table 17 Emotion Interference Task (EIT) accuracy and response time means (SEM) for each treatment combination (n=72; 36 FO, 36 OO).

		FO				OO			
		Stress		Non-Stress		Stress		Non-Stress	
		M	SEM	M	SEM	M	SEM	M	SEM
Accuracy	Negative	0.88	0.034	0.91	0.03	0.84	0.03	0.91	0.03
	Neutral	0.91	0.025	0.94	0.02	0.87	0.03	0.89	0.02
	Scrambled	0.91	0.025	0.91	0.03	0.87	0.03	0.89	0.03
Response Time	Negative	1460	61.24	1433	64.21	1461	61.24	1455	64.21
	Neutral	1455	59.96	1426	63.79	1466	59.96	1444	63.79
	Scrambled	1496	58.05	1459	59.86	1445	58.05	1464	59.86

Stress reduced accuracy ($p<.05$). No other main effects or interactions were found for accuracy or response time (all $ps>.2$).

Table 18 Morphed Faces Task (MFT) rated intensity means (SEM) for each treatment combination (n=72; 36 FO, 36 OO).

	FO				OO			
	Stress		Non-Stress		Stress		Non-Stress	
	M	SEM	M	SEM	M	SEM	M	SEM
Happy	2.94	0.29	2.85	0.25	3.12	0.26	3.09	0.25
Anger	5.07	0.19	5.17	0.15	5.05	0.16	5.08	0.15
Sad	2.75	0.15	2.78	0.15	2.53	0.15	2.44	0.15
Surprise	2.80	0.24	2.10	0.23	4.26	0.16	4.12	0.15
Fear	4.53	0.16	4.49	0.15	4.26	0.16	4.12	0.15
Disgust	2.91	0.25	2.78	0.24	2.96	0.25	2.96	0.24

Rated intensity differed as a function of emotion (p<.001; angry > fear > happy > disgust > surprise > sad). No other main effects or interactions were found (all ps >.30).

Table 19 Morphed Faces Task (MFT) stop frames means (SEM) for each treatment combination (n=72; 36 FO, 36 OO).

	FO				OO			
	Stress		Non-Stress		Stress		Non-Stress	
	M	SEM	M	SEM	M	SEM	M	SEM
Happy	18.31	2.43	15.96	2.05	19.80	2.43	18.49	2.05
Anger	67.55	2.82	68.81	2.71	67.35	2.82	69.26	2.71
Sad	87.21	2.19	87.69	1.73	85.17	2.19	88.30	1.73
Surprise	24.32	2.58	23.85	2.35	24.23	2.58	24.03	2.35
Fear	77.00	2.72	76.71	2.63	76.38	2.72	78.82	2.63
Disgust	15.70	2.42	16.00	1.83	17.64	2.42	15.98	1.83

Stop frames differed as a function of emotion (p<.001; disgust < happy < surprise < anger < fear < sad) wherein lower stop frames indicate higher sensitivity to emotional expression. No other main effects or interactions were found (all ps >.30).

Table 20 Cognitive Reappraisal Task (CRT) ratings of unpleasantness means (SEM) for each treatment combination following the non-stressful Trier Social Stress Test (n=72; 36 FO, 36 OO).

	FO		OO	
	M	SEM	M	SEM
Reappraise	4.16	0.17	4.07	0.17
Maintain	4.98	0.16	4.80	0.16

Images were rated as more unpleasant during the maintain than reappraisal condition (p<.001) No other main effects or interactions were found for (all ps>.53).

FIGURES

Figure 1 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 completed the stressful or non-stressful Trier Social Stress Test (TSST), followed by the Cognitive Reappraisal Questionnaire (CRQ), POMS, STICSA-S, and saliva (post-supplementation only). They then completed the Emotional Interference Task (EIT), Morphed Faces Task (MFT), and Cognitive Reappraisal Task (CRT; post-supplementation only) and final saliva sample.

Figure 2 Rated confusion increased during the stress condition and decreased during the control condition in the OO group ($p<.001$) but remained relatively stable in the FO ($p=.100$) group.

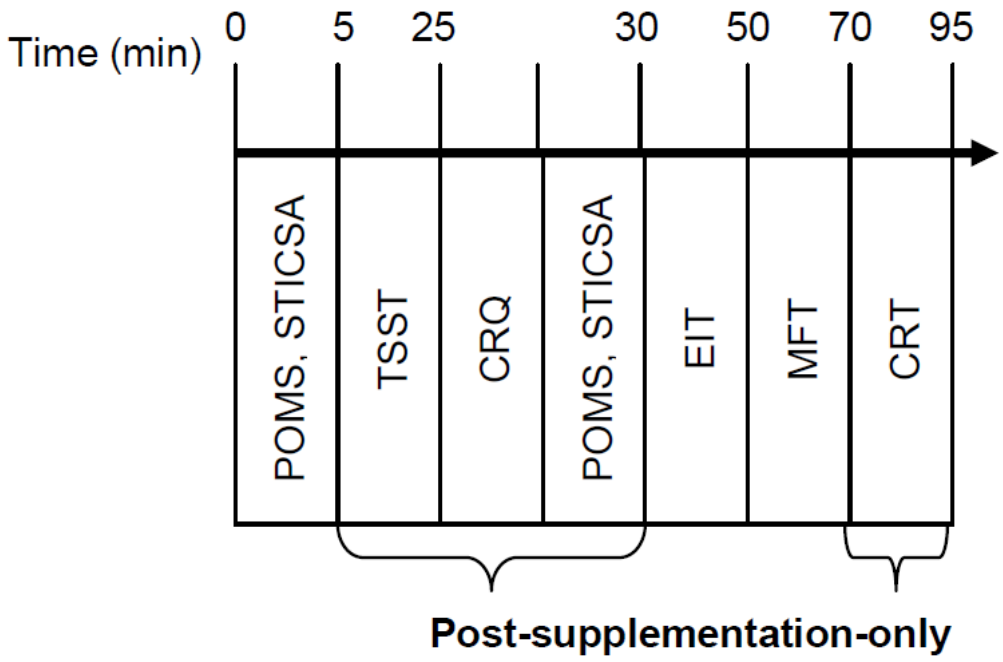
Figure 3 Rated somatic anxiety was lower in the control than stressful condition in the FO ($p<.001$) but not OO ($p>.42$) group.

Figure 4 Reappraisal was used more suppression during the stressful ($p<.001$) but not non-stressful ($p=>.19$) Trier Social Stress Test (TSST).

Figure 5 Heart rate was higher during the stressful than non-stressful Trier Social Stress Test (TSST; $p=<.01$) but not before ($p>.98$) or after ($p>.35$) TSST.

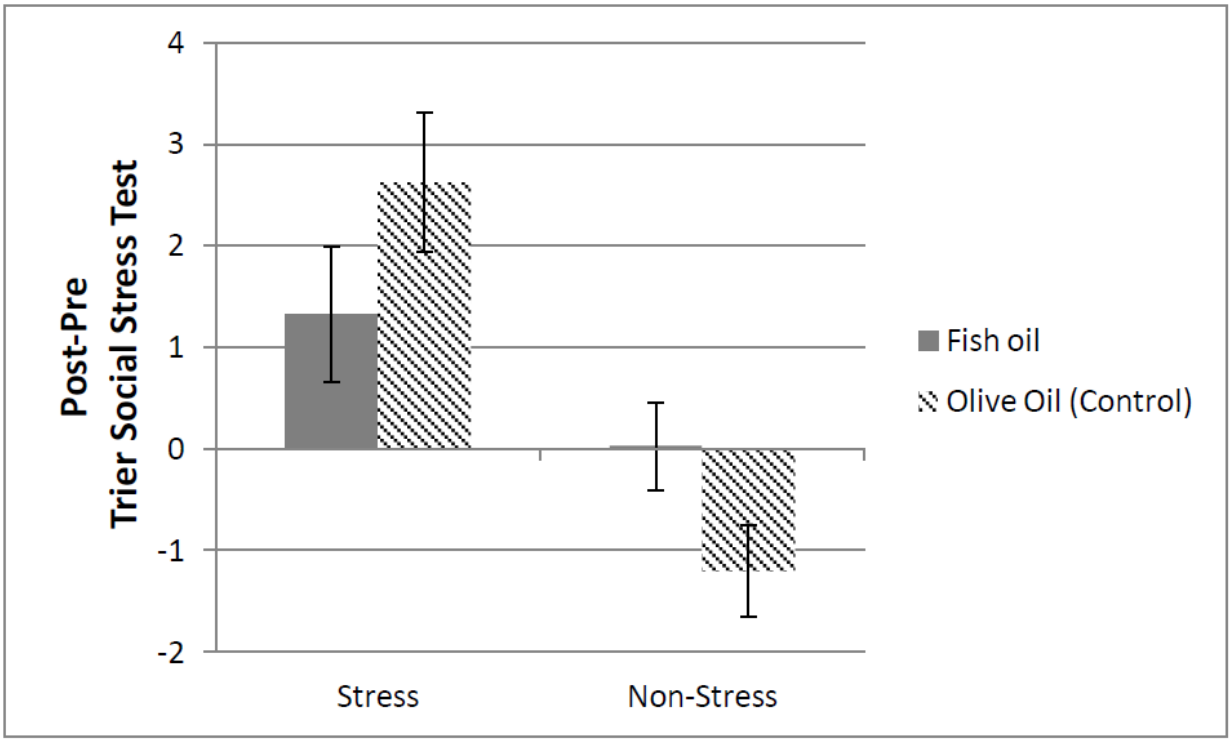
[Figure 1]

SCHEMATIC REPRESENTATION OF STUDY SCHEDULE



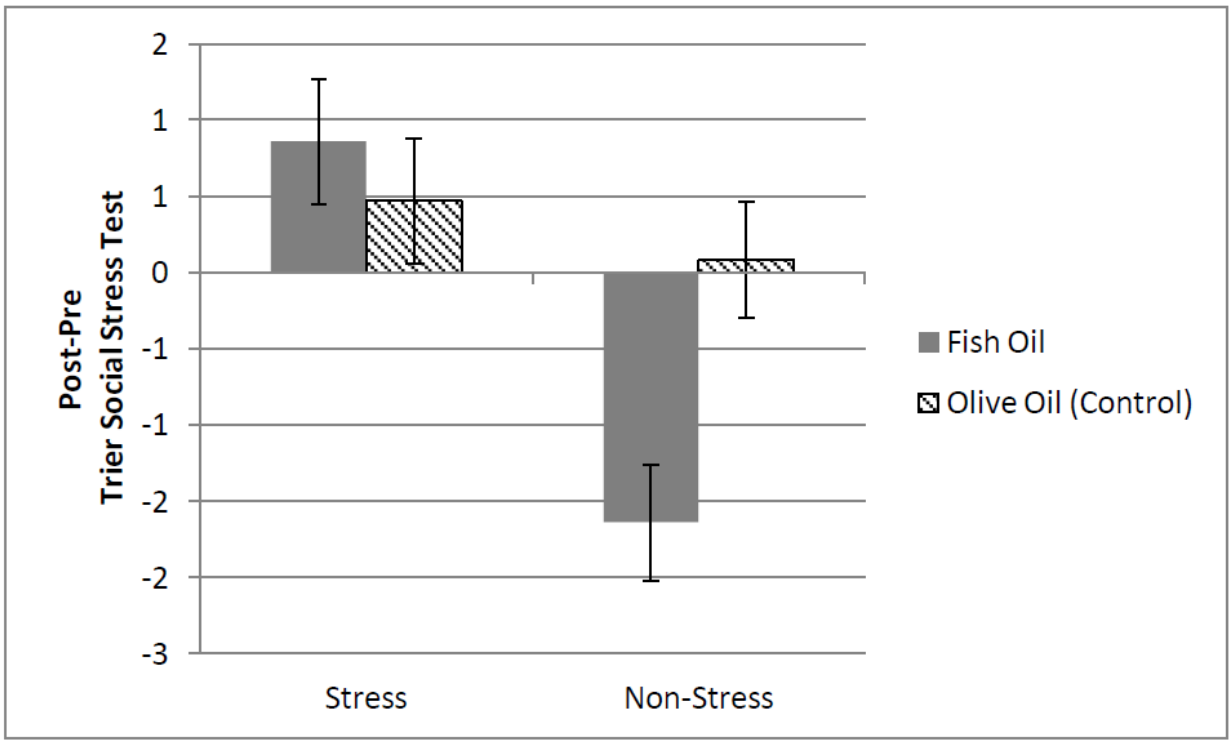
[FIGURE 2]

EFFECT OF FISH OIL AND OLIVE OIL ON RATED CONFUSION



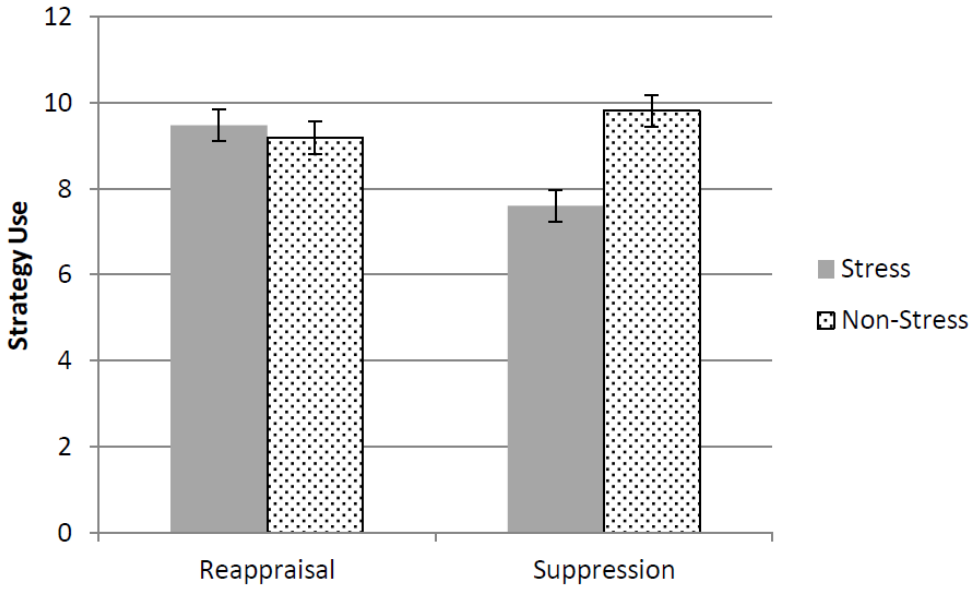
[FIGURE 3]

EFFECT OF FISH OIL AND OLIVE OIL ON RATED SOMATIC ANXIETY

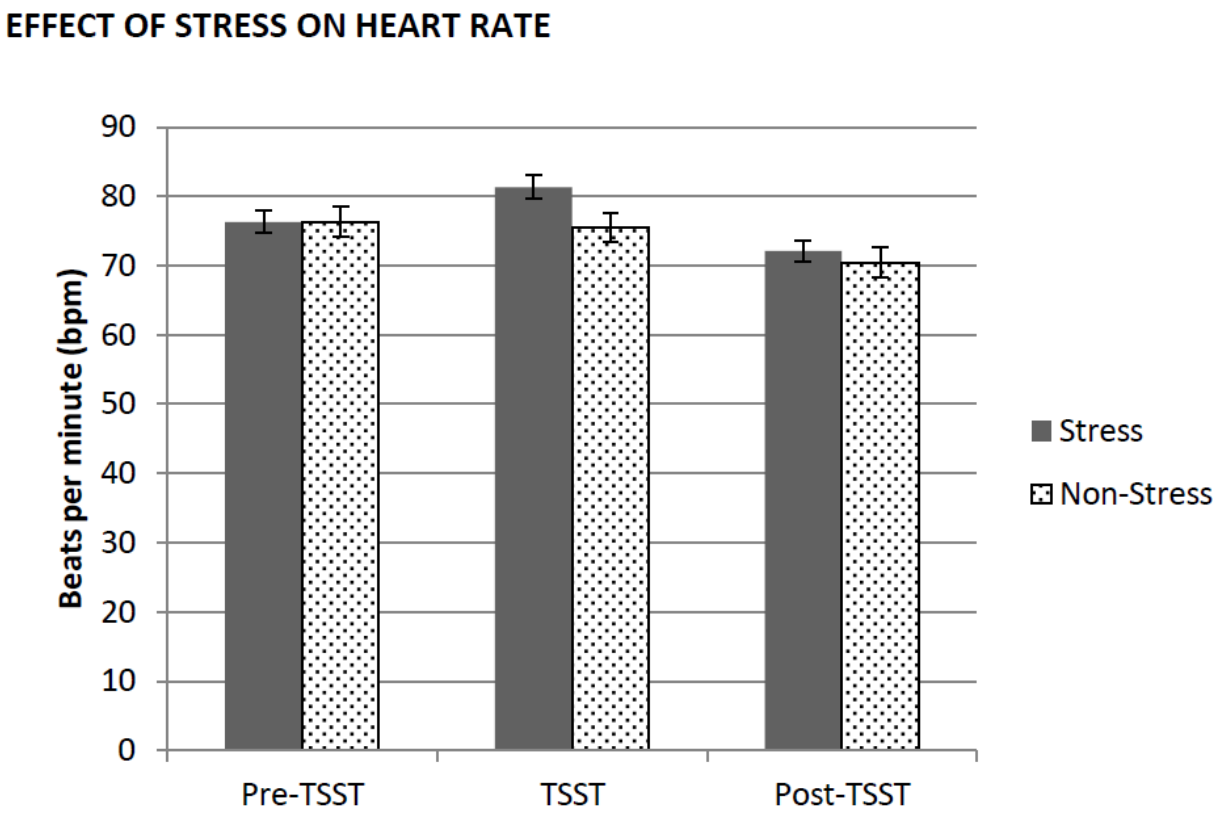


[FIGURE 4]

EFFECT OF STRESS ON REAPPRAISAL AND SUPPRESSION UTILIZATION



[FIGURE 5]


EFFECT OF STRESS ON HEART RATE
Beats per minute (bpm)
90
80
70
60
50
40
30
20
10
0
Pre-TSST
TSST
Post-TSST
Stress
Non-Stress