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# Polyunsaturated Fatty Acids: Health, the Brain, and the Human Diet

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## Abstract

Omega-3 Polyunsaturated Fatty Acids (n-3 PUFA) are essential for human health and development. N-3 PUFA researchers have linked n-3 PUFA deficiency to several leading causes of American deaths (Kung, Hoyert, Xu, & Murphy, 2008), including cardiovascular disease (Oomen et al., 2000), cancer (Ge et al., 2002), cerebrovascular accidents (Iso et al., 2001), diabetes mellitus (Gillen, 2005), and Alzheimer's disease (Morris et al., 2003). Developmentally, researchers have reported that infants with higher n-3 PUFA intakes perform significantly better on developmental tests compared to infants with lower n-3 PUFA intakes (Carlson et al., 1992; Daniels, Longnecker, Rowland, & Golding, 2004). Additionally, n-3 PUFA have been observed as efficacious in treating and preventing mood disorders (Stoll et al., 1999; Williams et al., 2006), attention deficit disorder (Gadoth, 2008), and autism (Amminger et al., 2007; Bell et al., 2004). In this study, we investigated the cognitive benefits of n-3 PUFA on healthy, college-aged individuals. Participants completed a food frequency questionnaire (FFQ) designed to measure n-3 PUFA. Low n-3 PUFA consumers (n=31) and high n-3 PUFA consumers (n=32) completed three cognitive assessments: a digit-span test, a Stroop Test, and a Trail Making Test. A series of t-tests and ANOVA tests indicated that there were no significant differences in the above cognitive measures as a function of self-reported n-3 PUFA intake (i.e., high or low). These results could have derived from participant error in reporting n-3 PUFA intake. As well, the FFQ scoring could have misattributed the n-3 PUFA values of certain foods. Though these results indicate that there were no cognitive benefits associated with high n-3 PUFA consumption, the cognitive benefits of n-3 PUFA require more research across populations of different ages.

# Polyunsaturated Fatty Acids: Health, the Brain, and the Human Diet

At the conclusion of the twentieth century, American consumers became increasingly interested in health-oriented nutrition and lifestyle choices (Simopoulos, 2001a). This increased attention on health may have derived from the significant number of deaths from chronic diseases throughout American society, and such diseases have perpetuated into the twenty-first century (Kung et al., 2008). Though the scale of disease in America appears daunting at first, new research into human nutrition may have found a conventional solution to the array of problems that face American health. Throughout recent scientific research, scientists have become increasingly interested in the relationship between human health and the essential fatty acids (EFA), meaning the omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFA) which appear mutually necessary for positive human health. Within many studies on the EFA, researchers have repeatedly produced new and profound experimental results when incorporating n-3 PUFA into the human diet.

Researchers have linked n-3 PUFA deficiency to five of America's top ten causes of death (Kung et al., 2008): cardiovascular disease (CVD; Oomen et al., 2000), cancer (Ge et al., 2002), stroke (He et al., 2002; Iso et al., 2001), diabetes mellitus (Gillen, Tapsell, Patch, Owen, & Batterham, 2005) and Alzheimer's disease (Morris et al., 2003). Recent research has also focused on n-3 PUFA in the maternal and fetal diets and their respective benefits on prenatal and postnatal development. Researchers have reported that infants with higher n-3 PUFA intake perform significantly better on developmental tests compared to infants with lower n-3 PUFA intake (Carlson, Cooke, Werkman, & Tolley, 1992; Daniels et al., 2004). Despite their apparent benefits, n-3 PUFA have abruptly disappeared from the human diet after the agricultural revolution (Simopoulos, 2000). With humans evolving alongside a diet high in n-3 PUFA

(Simopoulos, 2006), the reintroduction of n-3 PUFA into the human diet could promote healthful development and curb disease worldwide.

*Fatty Acids and the Human Body*

*Biochemistry of the Fatty Acids*. Fatty acids typically have an even number of carbon atoms, ranging from 16 to 26 total carbon atoms along the carbon chain (Ruxton, 2007). Fatty acids with only single bonds are considered saturated fatty acids, while fatty acids with at least one double bond between carbon atoms are considered unsaturated; therefore, fatty acids with multiple double bonds between carbon atoms are labeled polyunsaturated, PUFA. Certain PUFA have piqued the attention of many researchers, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). DHA and EPA are both n-3 PUFA, with the omega value denoting the location of the first double bond along the carbon chain. DHA and EPA are synthesized from the n-3 precursor alpha-linolenic acid (ALA) (Crawford, 1992; Gadoth, 2008; Ruxton, 2007; Simopoulos, 2000).

Aside from n-3 PUFA, n-6 PUFA also have a critical place in the human diet. The n-6 PUFA arachidonic acid (AA) is synthesized from the precursor linoleic acid (LA) (Gadoth, 2008; Ruxton, 2007; Simopoulos, 2000). ALA and LA are essential for the human diet as neither is synthesized endogenously by humans (Ruxton, 2007). Additionally, the n-3 and n-6 families cannot be interconverted (Ruxton, 2007; Simopoulos, 2000). Mammalian cells cannot convert n-6 to n-3, as they lack the converting enzyme n-3 desaturase (Simopoulos, 2006). Therefore, both n-3 and n-6 PUFA must have a place in the diet in order for a human to receive the proper nutrients from each variety of fatty acid.

*Fatty acids in the central nervous system*. Fatty acids are key components to the brain, and Owen, Rees, and Parker (2008) suggested that DHA and EPA play an important role in the

central nervous system (CNS). Altogether, PUFA comprise 15% to 30% of the brain's dry weight (Hallahan & Garland, 2005). AA and DHA are the main fatty acids in the cell membrane phospholipids, comprising 6% of their dry weight, while LA, ALA and EPA have a much lower concentration in the nervous tissue (Gadoth, 2008). The PUFA composition of cell membranes depends significantly on dietary intake (Simopoulos, 2003; Simopoulos, 2006). Of the brain's structural material, 60% is lipid, composed of cholesterol and phosphoglycerides that are rich in fatty acids, primarily AA and DHA (Crawford, 1992). In the rods of the retina, DHA accounts for some 50% to 60% of the fatty acids used in the polar phosphoglycerides of the plates (Crawford, 1992; Kidd, 2007). Additionally, DHA has the potential to affect membrane fluidity and blood-brain barrier function, along with dopaminergic and serotoninergic transmission (Gadoth, 2008; Kidd, 2007; Owen, Rees, & Parker, 2008).

*Evolutionary Significance of PUFA*

Animal life likely evolved in an environment rich in n-3 PUFA, with a smaller amount of n-6 PUFA available when compared to modern times (Crawford, 1992). N-3 PUFA have existed within the diet of living things throughout evolutionary history and probably dominated the food chain until the end of the Cretaceous period (Crawford, 1992). Human genes today are very similar to the genes of human ancestors 40,000 years ago during the Paleolithic period, when the genetic profile of modern humans was established (Simopoulos, 2003). A dietary balance existed between n-3 and n-6 PUFA consumption for millions of years during the evolution of the genus *Homo*, and genetic changes occurred partly due to those dietary influences (Simopoulos, 2006).

According to paleontological studies, human ancestors had a diet rich in marine foods, which in turn contain significant levels of n-3 PUFA (Crawford, 1992). Studies investigating Paleolithic nutrition and modern hunter-gatherer populations suggest that humans evolved while

consuming a diet much lower in saturated fatty acids than modern diets and small but nearly equal amounts of n-3 and n-6 PUFA (Eaton & Konner, 1985; Simopoulos, 2001b). Before the agricultural revolution 10,000 years ago, humans ate a wide variety of wild plants, which are rich in n-3 PUFA (Simopoulos, 2001b), along with an equal amount of n-3 and n-6 PUFA (Simopoulos, 2000); but today 17% of plant species, specifically cereal grains, provide 90% of the world's food supply (Simopoulos, 2003). Cereal grains are high in carbohydrates and n-6 PUFA, but contain low levels of n-3 PUFA (Simopoulos, 2003). For 99.9% of humankind's history, humans rarely consumed cereal grains; therefore, humans have had less than 500 generations to adapt to a food type that provides both calories and protein to the majority of humanity (Simopoulos, 2003). The current Western diet has shifted, grossly increasing the dietary ratio of the EFA over the last 150 years, with n-3 to n-6 ratios estimated as high as 1:25 in the modern Western World (Innis & Jacobson, 2007; Simopoulos, 2000; Simopoulos, 2006).

Ruxton (2007) suggests that the dietary habits of human ancestors may explain the significant amount of DHA in the human nervous system, as this evolutionary diet supported high levels of DHA in the brain (Novak, Dyer, & Innis, 2008). Both n-3 and n-6 PUFA are required in the diet (Crawford, 1992) and the balance between them appears critically important (Yehuda, Rabinovitz, & Mostofsky, 2008), as n-3 and n-6 PUFA compete in the body, leading to a decreased secretion of DHA alongside an increased secretion of n-6 PUFA in plasma, with the body preferentially absorbing n-6 PUFA over DHA (Novak, Dyer, & Innis, 2008). Some results see this conflict from a different perspective, suggesting that EPA, an n-3 PUFA, may inhibit the conversion of LA to AA (Olafsdottir, Thorsdottir, Wagner, & Elmadfa, 2006). This perspective sees the n-3 PUFA preferentially absorbed and inhibiting the synthesis of the n-6 PUFA, again emphasizing the endogenous competition of the two varieties of fatty acids (Novak, Dyer, &

Innis, 2008). Nevertheless, the lopsided amount of n-6 PUFA in the modern diet may account for the influx of diseases and health problems throughout modern American society, as researchers have perceived health benefits within populations adhering to the evolutionary diet. The culture in Crete tends to consume a diet very similar to that of the Paleolithic period and their population has experienced lower levels of CVD (Simopoulos, 2001b). The evolutionary balance between n-3 and n-6 PUFA in the human diet may prevent disease and explain the perceived health benefits of people that maintain substantial amounts of n-3 PUFA in their diet.

*Diet and Disease*

A low ratio of n-3 to n-6 PUFA could potentially help prevent or manage chronic diseases (Simopoulos, 2006). However, advisory bodies on nutrition and food have yet to outright acknowledge the importance of PUFA, such as the Committee on Medical Aspects of Food Policy (COMA). In 1994, COMA declared that n-3 PUFA intake should be increased from 0.1 grams per day to 0.2 grams per day, which is still well below the suggested dosages of many studies examining the efficacy of n-3 PUFA in preventing and treating diseases (Ruxton, 2007). Aside from COMA, other advisory bodies have suggested alternative suggestions for dietary n-3 PUFA intake. The American Heart Association has suggested 1000 mg of DHA/EPA per day (Kidd, 2007). Contrarily, the American Diabetes Association (2008) has advocated for the consumption of two or more servings of fish per week. The dissonance between the suggestions of different organizations has provided little direction for consumers. Therefore, until policymakers and advisory bodies follow the evidence and create a consensus on necessary n-3 PUFA consumption rates, consumers themselves must become aware of the benefits of n-3 PUFA and construct appropriate diets for their individual health.

Many dietary sources for n-3 PUFA exist, and a responsible diet could be simplest path towards disease prevention. To obtain n-3 PUFA from dietary sources, many communities follow the evolutionary diet which focused on marine foods (Crawford, 1992). According to Oomen et al. (2000), fatty fish such as trout, mackerel, salmon, and tuna provide more DHA and EPA than white fish, resulting in a greater overall protection against CVD (Nettleton & Katz, 2005). As well, fish oils possess constituent n-3 PUFA such as DHA and EPA, and therefore fish oil supplementation could serve as a source of n-3 PUFA (Ruxton, 2007).

Other meat sources of n-3 PUFA include grass-fed beef (Howe, Meyer, Record, & Baghurst, 2006; Ponnampalam, Mann, & Sinclair, 2006; Winningham, 2009) and lamb meat (Howe et al., 2006), as lamb tend to be raised grass-fed. As well, n-3 fortified eggs provide an effective source of n-3 PUFA (Kidd, 2007; Ohman et al., 2008; Shapira, Weill, & Loewenbach, 2008; Winningham, 2009). For vegetarians, botanical sources of n-3 PUFA exist, such as walnuts (Gebauer, Psota, Harris, & Kris-Etherton, 2006; Gillen et al., 2005; Winningham, 2009) and flaxseed (Gebauer et al., 2006; Winningham, 2009). Another source of n-3 PUFA supplementation derives from flaxseed oil, which has experimentally observed some efficacious results (Barceló-Coblijn et al., 2008; Riediger et al., 2008).

Despite the availability of many foods containing n-3 PUFA, the support for n-3 PUFA in the diet has yet to gain significant popularity throughout the food industry. Historically, the health emphasis has come from avoiding saturated fatty acids rather than consuming n-3 PUFA; however, recent research on diseases has initiated a growing public awareness of n-3 PUFA and their benefits. Previously, attempts to limit CVD have focused primarily on saturated fatty acids and n-6 PUFA; however, recently the messages on diet include the positive effects of n-3 PUFA (Ruxton, 2007; Simopoulos, 2001b).

*N-3 PUFA and CVD*. Fish-eating communities consume higher levels of n-3 PUFA and see lower rates of CVD (Bulliyya, 2002; Crawford, 1992; Dewailly, Blanchet, Gingras, Lemieux, & Holum, 2003). Bulliyya (2002) compared fish-eating and non fish-eating South Indian villages and noticed that non-fish consumers showed a significantly higher risk for CVD. Crawford (1992) suggests that the fish eating communities of the Eskimos and the Japanese have lower death rates from CVD. Dewailly et al. (2003) noticed when comparing three ethnic groups in Quebec that the Inuit, who regularly consumed fish, presented the lowest CVD risk, even though the community had a high prevalence of both obesity and smoking. Yamagishi et al. (2008) surveyed 57,972 Japanese men and women and found that fish and n-3 PUFA consumption were associated with 18% to 19% lower risk of overall mortality from CVD. Other researchers have investigated whether n-3 PUFA (i.e., DHA/EPA) supplementation had a positive effect on heart health. Bucher, Henglster, Schindler, and Meier (2002) examined a ratio between DHA and EPA, experimenting in supplementation levels of 0.6 to 3.7 grams per day for DHA and 0.3 to 6.0 grams per day for EPA, concluding that n-3 PUFA could reduce overall mortality from either myocardial infarctions or sudden death from coronary heart disease.

The influence that n-3 PUFA has on preventing CVD could derive primarily from a balanced ratio between n-3 and n-6 PUFA rather than any unique properties of n-3 PUFA. The higher levels of n-3 PUFA in the diet may simply offset the already prevalent levels of n-6 PUFA in the modern diet. Evidence indicates a tandem development of the vascular and nervous systems, and that a balanced ratio between n-3 and n-6 PUFA must be present in order for both systems to function optimally (Crawford, 1985). Therefore, a proper ratio between n-3 and n-6 PUFA throughout a population should lead to a decrease in the prevalence of CVD. Simopoulos

(2006) suggested that a ratio of 1:4 n-3 to n-6 PUFA was effective at preventing secondary CVD and decreased total mortality by 70%.

*N-3 PUFA and diabetes mellitus*. N-3 PUFA have become increasingly popular in the treatment of diabetes mellitus, a close associate of CVD. Nettleton and Katz (2005) reviewed an array of literature focused on n-3 PUFA and diabetes, ultimately concluding on an association between the two. In studies involving significant levels of fish or n-3 PUFA consumption, the results illustrate a relationship between fish consumption and a reduced prevalence of Type 2 diabetes and CVD (Nettleton & Katz, 2005). The American Diabetes Association (2008) has advocated for the consumption of two or more servings of non-commercially fried fish per week as a source of n-3 PUFA to help manage diabetes.

Japanese islanders consume more n-3 PUFA than Japanese mainlanders, and the islanders have a lower prevalence of Type 2 diabetes compared to the mainlanders (Kagawa et al., 1982). Examining diabetes more experimentally, Gillen et al. (2005) found that the consumption of walnuts, high in n-3 PUFA, allots optimal proportions of fat intake without adverse effects in patients with diabetes mellitus. However, not all studies have found efficacy in the use of n-3 PUFA to treat or prevent diabetes. In a longitudinal study among older Iowan women, n-3 PUFA consumption was positively associated with incidences of diabetes over an 11 year period (Meyer, Kushi, Jacobs, & Folsom, 2001). Incongruent results have characterized the relationships between various studies investigating the therapeutic value of n-3 PUFA. Still regardless of counterintuitive and disparate findings, as researchers continue to discover more aspects of the EFA, they will hopefully come closer to fully utilizing the therapeutic functions of n-3 PUFA.

*N-3 PUFA and inflammatory diseases*. Some degree of efficacy has been observed in the use of fish oils and n-3 PUFA to treat multiple inflammatory diseases, including inflammatory bowel disease, asthma, cystic fibrosis, and rheumatoid arthritis (Ruxton, 2007). In studies examining rheumatoid arthritis, a variety of improvements have been observed. Fortin et al. (1995) reported significant reductions in the duration of morning stiffness and the number of tender joints after three months of pharmacotherapy using fish oil supplementation. As well, Mehta, Dworkin, and Schwid (2009) review research investigating the effects of n-3 PUFA on multiple sclerosis, examining the efficacy of the anti-inflammatory qualities of n-3 PUFA on the autoimmune inflammatory disorder.

Certain biological processes could explain the relationship between n-3 PUFA and inflammation regarding certain diseases. Ingestion of DHA and EPA from fish oil leads to a diminished formation of leukotriene $B_{4,}$ which is an inducer of inflammation, but also increased levels of leukotriene $B_5$, which is a weak inducer of inflammation (Simopoulos, 2000; Simopoulos, 2003). Perhaps the effects of DHA/EPA on both types of leukotriene stabilize the inflammatory processes, and in turn reduces the effects of inflammatory diseases. As researchers continue to discover the endogenous processes of n-3 PUFA, the underlying biology of their benefits gradually comes to light, demonstrating how a healthy human life depends on a healthy diet.

*N-3 PUFA and cancer*. Some researchers have found benefits of n-3 PUFA on cancer, demonstrating the spectrum of effects that the nutrients have on human health. Simopoulos (2003) cites studies investigating the relationship between a low n-3 to n-6 PUFA ratio and cancer. Ge et al. (2002) provided normal rat cardiomyocytes and human breast cancer cells with the enzyme n-3 desaturase, gathering the enzyme from the roundworm *caenorhabitis elegans*, as

mammillian cells lack this enzyme that converts n-6 to n-3 PUFA (Simopoulos, 2006). With the n-3 desaturase enzyme present, Ge et al. (2002) observed the ratio of n-3 to n-6 PUFA lower to a nearly 1:1 ratio. Thereafter, cancer cells expressing the enzyme n-3 desaturase with the low n-3 to n-6 PUFA ratio underwent apoptotic death, whereas the control cancer cells with a high n-3 to n-6 PUFA ratio continued to proliferate (Ge et al., 2002). Donaldson (2004) also suggested that an imbalance of n-3 to n-6 PUFA contributes to an increased risk of cancer, and suggests a diet with consideration for a low n-3 to n-6 PUFA ratio. Similarly, Fradet, Cheng, Casey, and Witte (2009) found a preventative effect of n-3 PUFA against aggressive prostate cancer and advocated for high dietary n-3 PUFA intake. As diseases continue to propagate throughout modern society, the successes of n-3 PUFA in supporting good health present a potentially simple and preventative method for many far-reaching diseases.

*Diet and the developing brain*

Aside from the biology of diseases, n-3 PUFA have distinct effects on the biology of the brain. N-3 PUFA have an important connection to a variety of cognitive functions. Specifically, n-3 PUFA have a direct influence on neural-cognition, regulating neurotransmitters and assisting with fluidity in neuronal membranes (Gadoth, 2008; Yehuda, Rabinovitz, & Mostofsky, 1999). Aside from their effects on the mature brain, n-3 PUFA have an essential connection to cognitive development (Gadoth, 2008). N-3 and n-6 PUFA are both required for proper brain development and the function of the mature brain through their effects on cell membrane structures and electrophysiology (Kitajka et al., 2004).

*The physiology of PUFA in the developing brain*. According to Dobbing (1972), brain development is dependent on nutrition in the critical stages of growth. Fatty acid composition of human milk depends on both the short-term and long-term maternal diet (Joordens, Kuipers,

Muskiet, & Robson, 2005). Thus, the DHA and EPA in breast milk can biologically vary among samples (Smit, Martini, Mulder, Boersma, & Muskiet, 2002). Novak, Dyer, and Innis (2008) implied that the early diet could potentially render the amount of DHA and n-6 PUFA in the infant brain of humans. Evidence shows the dependence of infant and fetal DHA totals on the dietary intake of DHA by the mother, which demonstrates a possible requirement of n-3 PUFA in the diets of pregnant and lactating women to support the visual and neural development of their infants (Innis, 2003). Gustafsson, Duchén, Birberg, and Karlsson (2004) advocated for high PUFA levels in breast milk for the proper cognitive development of infants. However, according to Demmelmair, Baumheuer, Koletzko, Dokoupil, and Kratl (1998), only 30% of human milk fatty acids derive directly from the maternal diet, while the mother's stores provide the major portion. Nevertheless, n-3 PUFA appear critical for brain development and the fetus or infant depends on the mother to receive the proper amount of essential nutrients.

The accretion of n-3 and n-6 PUFA increases progressively during the final trimester of a pregnancy, demonstrating a possible requirement of such acids in the system (Clandinin et al., 1980). According to Clandinin, Chappell, and Van Aerde (1989), during the third trimester of pregnancy, fetuses require approximately 40 milligrams to 60 milligrams of n-3 PUFA per kilogram body weight per day. Crawford, Hassam, and Williams (1976) suggest that both AA and DHA have an important developmental role in the CNS of mammals. Helland, Smith, Saarem, Saugstad, and Drevon (2003) suggested that the last trimester and first postnatal months experience a growth spurt in the human brain, with a large increase in cerebral content of AA and DHA. During intrauterine and postnatal growth, significant amounts of AA and DHA accumulate in various tissues and specifically the brain (Clandinin, Chappell, & Van Aerde, 1989).

*The developmental benefits of n-3 PUFA.* DHA appears to be crucial for normal cognitive development and functions, as any deviation from its physiological level is associated with cognitive impairments or deficits (Yehuda, Rabinovitz, & Mostofsky, 1999; Yehuda, Rabinovitz, & Mostofsky, 2008). Helland et al. (2003) observed a relationship between intelligence scores at four years of age and the concentration of DHA in the plasma phospholipids. Low PUFA status correlated with adverse neurodevelopment during the first four postnatal months, especially in preterm infants, as demonstrated in visual, perceptive, cognitive, and motor development tests (Innis, 2003; Koletzko et al., 2001; Larque, Demmelmair, & Koletzko, 2002). AA has an important role in neural-brain development, and PUFA intake in the last trimester of pregnancy may have an effect on myelin formation and maintenance (Parra-Cabrera, Moreno-Macias, Mendez-Ramirez, Schnaas, & Romieu, 2007). Yehuda, Rabinovitz, and Mostofsky (2008) suggested that n-3 PUFA deficiency affected the rate of myelination. With myelin facilitating the speed at which impulses propagate along a myelinated fiber (Carlson, 2008; Georgief, 2007), the effects of n-3 PUFA on myelination may explain the perceived cognitive benefits of many developmental experiments focusing on n-3 PUFA.

Daniels et al. (2004) found that mothers consuming fish four times a week during pregnancy had babies with higher developmental scores than non-fish consumers at 18 months after birth. Supplementing preterm formula with fish oil led to better visual function (Carlson et al., 1992). At the age of four, children of mothers supplemented with 1.18 grams of DHA and 0.8 grams of EPA per day through cod liver oil during pregnancy from week 18 until delivery had significantly higher IQ than children of mothers supplemented with corn oil (Helland et al., 2003). More studies need to investigate the benefits of n-3 PUFA during development on

cognitive skill sets, as some counterevidence to this hypothesis has existed in the field (e.g., Ruxton, 2007).

According to Colombo (2001), many studies have found benefits of n-3 PUFA in the diet during development and some studies have observed no significant effects, but Koletzko et al. (2001) assuredly argued that no study has found developmentally detrimental effects of infant formulas containing both DHA and AA. Contrarily, Church, Jen, Jackson, Adams, and Hotra (2009) measured the auditory brainstem response of rats to assess brain development and sensory function. Church et al. (2009) found that both deficient and excess amounts of n-3 PUFA in the maternal diet during pregnancy and lactation led to adverse neurological development among rat pups. The excess group experienced more significant deficits in the auditory brainstem response measurement than the deficient group, with 19% of the excess n-3 PUFA rats developing an early-adulthood onset of neurological deficits (Church et al., 2009). These results express the necessity of balance when consuming the EFA, as excess levels of either can impede on development. As long as a maternal diet appropriates reasonable amounts of both n-3 and n-6 PUFA, brain growth should proceed without any developmental deficits.

*Diet in pathologies and cognitive disorders*

N-3 PUFA also appear to affect cognition throughout life. Fontani et al. (2005) explains that n-3 PUFA may affect many pathways of neurotransmission, including dopamine acetylcholine, norepinephrine, and serotonin. These effects may explain the observed effects of n-3 PUFA on multiple components of cognition, from mood to learning (Fontani et al., 2005). As well, animals with diets high in n-3 PUFA have a greater abundance of neurotransmitters, a lower amount of damage to their neurons, and better growth in the region of the brain associated with memory—the hippocampus (Winningham, 2008). All these effects on the CNS may

contribute to the observed effects of n-3 PUFA on mood, attention, memory and overall brain health.

*N-3 PUFA and age-related cognitive decline*. Aside from their developmental benefits, scientists have also observed the efficacy of n-3 PUFA in treating dementia and the cognitive declines that occur in the latter part of the lifespan. Such diseases and pathologies have significant effects on society, as Alzheimer's disease took the lives of 72,000 people in 2005 (Kung, et al., 2008). Dementia and Age-related cognitive decline, along with all associated diseases and disorders, have had significant impacts on the lives of many Americans, especially the elderly. After the age of sixty-five, incidences of Alzheimer's disease double across the population every five years, with half of the population over one-hundred years of age afflicted with the disease (Czech, Tremp, & Pradier, 2000). No cure for Alzheimer's disease exists, but some studies have found ways to avoid the onset of cognitive decline by changing the diet (Boudrault, Bazinet, & Ma, 2009).

A proper balance between n-3 and n-6 PUFA should improve mental health according to Simopoulos (2000). In turn, n-3 PUFA have developed a reputation of improving an array of mental disorders or diseases. N-3 PUFA appear to have significant benefits for older-aged people facing cognitive decline. Age-related cognitive decline and its associated diseases and disorders have been fought through the use of n-3 PUFA. Large scale epidemiological studies have associated fish consumption with a level of protection against dementia (Ruxton, 2007). Elderly people consuming fish at least once a week have a 60% lower risk of developing Alzheimer's disease over a 4-year period (Morris et al., 2003). Frequent fish consumption during midlife was associated with better semantic memory and cognitive function in later life, along with an avoidance of developing mild cognitive impairment (Eskelinen et al., 2008). In an 8.5 year

longitudinal study, Solfrizzi et al. (2006) noticed that individuals with a typical Mediterranean diet, high in n-3 PUFA, had significantly better cognitive performances after the 8.5 years when compared to individuals consuming less n-3 PUFA. As well, Solfrizzi et al. (2006) noticed that high n-3 PUFA intake protected against age-related cognitive decline.

*N-3 PUFA and cerebrovascular accidents.* Investigating another leading cause of American deaths (Kung et al., 2008), some researchers have concluded that n-3 PUFA could help prevent the prevalence of cerebrovascular accidents, including both ischemic and thrombotic strokes. In a large study on a female nurse population in the United States, Iso et al. (2001) found that fish consumption was associated with a reduced risk of thrombotic strokes, but also found that fish consumption was not associated with the risk of hemorrhagic strokes. Similarly, He et al. (2002) found an association between men consuming fish at least once to thrice a month and a reduced likelihood of ischemic strokes. Much like Iso et al. (2001), He et al. (2002) found no association between fish consumption and a reduced risk for hemorrhagic stroke. Yamagishi et al. (2008) studied a large population of Japanese men and women, and found no protective properties of n-3 PUFA against stroke. The incongruence between studies suggests that more research must investigate n-3 PUFA and their relationships to the various types of strokes, but preliminary results imply that n-3 PUFA could have potential for preventing strokes among some populations.

*N-3 PUFA and neurobehavioral developmental disorders*. Researchers have also observed links between n-3 PUFA and various pathologies or cognitive disorders that afflict people throughout their lifespan. Some efficacy may exist for n-3 PUFA in the treatment of Attention Deficit/Hyperactivity Disorder (ADHD). Richardson and Puri (2002) found that children with ADHD supplemented with an EFA combination improved significantly in their

attention and decreased their disruptive behaviors when compared to a group supplemented with olive oil. However, some researchers are still not entirely certain regarding the actual efficacious properties of PUFA in treating ADHD (Gadoth, 2008).

Alongside ADHD, many studies have investigated the relationship between n-3 PUFA and autism spectrum disorder. Researchers have readily investigated the contributing mechanisms to the disorder, which range from genetic predispositions to inadequate diet and nutrients (Amminger et al., 2007; Bejerot & Humble, 2008; Newschaffer et al., 2007; Vancassel et al., 2001). Vancassel et al. (2001) examined the plasma levels of a group of participants with autism and found significantly lower levels of n-3 PUFA without a decrease in n-6 PUFA, resulting in a significant increase in the ratio between the two varieties of PUFA.

To treat autism, researchers have also begun to experimentally assess the efficacy of n-3 PUFA. Amminger et al. (2007) supplemented children with autism with 1.5 grams of n-3 PUFA per day. The study found that when compared to a placebo group, children with autism supplemented with n-3 PUFA showed significant improvement in hyperactivity and stereotypy. However, Amminger et al. (2007) experienced shortcomings in their methodology, as they did not examine plasma concentrations of the PUFA in their participants, which Vancassel et al. (2001) emphasized as having such an important relationship with autism spectrum disorder.

Contrarily, Bell et al. (2004) did examine plasma concentration of PUFA among participants with autism supplemented with n-3 PUFA, finding increased levels in the body after supplementation. Bell et al. (2004) surveyed parents with children that suffered from autism. These parents supplemented their children with n-3 PUFA and they reported improved general health, sociability and cognitive skills, alongside decreased aggression and hyperactivity. Itomura et al. (2005) had similar findings in typically developing children, finding decreased

reported aggression in girls consuming n-3 PUFA fortified foods. Politi et al. (2008) found no efficacy in treating the pathological symptoms of young adults with autism through fish oil supplementation. Researchers examining the relationship between autism spectrum disorder and n-3 PUFA have generally observed a potential for n-3 PUFA in ameliorating some symptoms of autism, but future researchers must continue to investigate the validity of this assessment.

*N-3 PUFA and mood disorders*. Multiple studies have also examined n-3 PUFA and their relationship with depression and mood disorders, and – much like the studies on autism – they too have found mixed results. A series of studies have found convincing results regarding the mood-stabilizing capabilities of n-3 PUFA. Researchers have demonstrated an efficacy of n-3 PUFA in treating bipolar disorder in comparison to a placebo group when tested among thirty participants with bipolar disorder over four months (Stoll et al., 1999). Williams et al. (2006) found that low plasma concentrations of the EFA correlated with increased depression. According to McNamara et al. (2007), patients with nonpsychotic depression had lower levels of DHA in the orbitofrontal cortex at autopsy when compared with control individuals of the same age. Parker et al. (2006) found that n-3 PUFA may work therapeutically in the treatment of mood disorders. These results could explain the relationship between CVD and depression, as n-3 PUFA works therapeutically for both afflictions (Parker et al., 2006).

Though many studies have endorsed n-3 PUFA as effective in treating mood disorders, many studies have counterintuitive results. Freeman et al. (2008) and Miyake et al. (2006) found that n-3 PUFA had no efficacy in treating perinatal depression. Hakkarainen et al. (2004) found no association between mood scores and reported dietary fish consumption. Interestingly, one study found that too much n-3 PUFA – along with too little – could contribute to a depressed mood. According to Appleton et al. (2007), very high and very low consumptions of n-3 PUFA

was associated with high depressed mood scores. Despite somewhat high consumption rates of fish associating with lower depressed mood scores, the extremes of n-3 PUFA consumption rates demonstrated identical results (Appleton et al., 2007).

*Considering efficacy and risk*

Researchers have demonstrated n-3 PUFA as preventative and therapeutic in regards to a variety of diseases and pathologies. Additionally, researchers have linked n-3 PUFA to proper development, demonstrating a requirement of both EFA for proper cognitive functionality. Still, despite the array of observed benefits, some may argue that certain risks pertaining to n-3 PUFA consumption may outweigh the benefits.

*Frequent fish consumption and mercury poisoning*. N-3 PUFA advocates would commonly support the consumption of fish as essential for the healthy diet, but recently the concern of mercury poisoning has deterred many people from incorporating fish in their diet (Gadoth, 2008; Ruxton, 2007). However, educated consumers may reduce the risk of mercury poisoning by following the guidelines of the United States Food and Drug Agency (USFDA). The USFDA lists the mercury concentrations in species of fish on their website, warning consumers of hazardous levels (USFDA, 2001). According to Oomen et al. (2000) fatty fish such as trout, mackerel, salmon, and tuna provide sufficient DHA, and all of these listed fish contain lower levels of mercury according to the USFDA (2001). Therefore, mercury may be a negligible concern when consuming a safe species of fish with sufficient n-3 PUFA.

*N-3 PUFA and lipid peroxidation*. Aside from mercury, a common concern with n-3 PUFA derives from the risk of lipid peroxidation when administered without antioxidants (Ruxton, 2007; Wong et al., 1987). Olafsdottir et al. (2006) observed lipid peroxidation being higher in Icelandic women supplemented with cod liver oil than women not supplemented with

cod liver oil, but Olafsdottir et al. did not determine this difference to be significant and suggested that other factors that increase free radicals – such as smoking – may be more important. Still Amminger et al. (2007) and Politi et al. (2008) took precautions and administered participants with Vitamin E in addition to n-3 PUFA in order to avoid lipid peroxidation during their research. Kidd (2007) emphasized that DHA and EPA will not act as free radicals when in the presence of antioxidants, despite their highly unsaturated bonds.

Evolutionary history shows the human diet developing with an appropriate intake of antioxidants alongside the consumption of an equal amount of n-3 and n-6 PUFA (Simopoulos, 2001b; Simopoulos, 2006). Therefore, with antioxidants incorporated into the evolutionary diet, the benefits of n-3 PUFA may also require the presence of proper antioxidants, and Ruxton (2007) wrote that lipid peroxidation could explain why some studies regarding n-3 PUFA found confounding or opposite results to what prior research had previously found. However, when Politi et al. (2008) administered n-3 PUFA alongside Vitamin E to avoid lipid peroxidation, researchers still found no benefit of n-3 PUFA on the symptoms of autism spectrum disorder.

The place of Vitamin E in a marine diet remains a topic of debate, as Wander and Du (2000) deemed supplemental Vitamin E unnecessary. Their findings suggested that the intake of fish oil did not lead to increased oxidation, and therefore Vitamin E did not counter any harmful effects of n-3 PUFA. However, other researchers have suggested that Vitamin E and fish oil are complementary. Nair et al. (1993) found that participants excreted more peroxidation products within their urine when consuming more fish oil. These peroxidation products reduced by half when participants included Vitamin E in their diet. Therefore, researchers must come to a consensus on the nutritional relationship between Vitamin E and n-3 PUFA. Vitamin E may be

essential or unneeded, but – as of yet – no research has indicated any harm from simultaneous consumption of the two nutrients.

*Disparate results in studies on n-3 PUFA.* Much like the uncertainty surrounding the significance of lipid peroxidation, researchers have repeatedly found mixed results on the observed benefits of n-3 PUFA. Many confounding studies have demonstrated counterintuitive results to the established conceptions of n-3 PUFA (Ruxton, 2007). Mood disorder studies regarding n-3 PUFA have observed both benefits and no improvements in affective symptoms (Appleton, 2007; Freeman et al., 2008; Hakkarainen, 2004; Miyake et al., 2006; Parker et al., 2006; Stoll et al., 1999; Williams et al., 2006). Owen, Rees, and Parker (2008) suggest that co-varying dietary and lifestyle factors may account for the relationship between n-3 PUFA and depression.

Many of the n-3 PUFA studies on disorders and diseases affecting the CNS have also contained counterintuitive results. Though schizophrenia has been theoretically linked to PUFA deficiency according to Saugstad (1999), patients with schizophrenia consumed significantly more saturated fatty acids and PUFA than controls in a study by Strassnig, Brar, and Ganguli (2004). High levels of fatty acids in the brain have been observed as leading to the symptoms of Alzheimer's disease (Sanchez-Mejia et al., 2008). Helland et al. (2003) explained that researchers have observed enhanced visual acuity, problem solving, and neurologic development in infants consuming formulas with n-3 and n-6 PUFA; however, other studies have observed no such effects in infants.

Colombo (2001) cited an assortment of studies perceiving a positive effect or no effect at all of n-3 PUFA on infant cognitive performances. Koletzko et al. (2001) cites that no study has found developmentally detrimental effects of PUFA in infant formula. However, Church et al.

(2009) has observed developmentally detrimental effects of both deficient and excess n-3 PUFA in the maternal diet during pregnancy and lactation. Still the majority of studies have directed researchers towards a variety of therapeutic and preventive values to n-3 PUFA in an array of pathologies and diseases. Despite occasionally incongruent results, researchers have generally considered n-3 PUFA essential for the human diet and an effective nutrient for both body and brain health.

*The optimal dosage and ratio of PUFA.* Just as researchers have yet to entirely understand the benefits of n-3 PUFA, researchers have yet to agree on an optimal dosage of n-3 PUFA for therapeutic purposes. Just as Church et al. (2009) illustrated, both an excess and deficient amount of n-3 PUFA could potentially produce adverse effects. Therefore, researchers have experienced difficulty in determining an optimal dose. Simopoulos (2006) suggests that the therapeutic dosage of n-3 PUFA still depends on the severity of the disease as a result of genetic predisposition. Nothing is known yet regarding the optimal dosage ratio of n-3 to n-6 PUFA when given separately or given together in a preparation containing both compounds (Gadoth, 2008). However, the balance between n-3 and n-6 PUFA in the body is vital (Ruxton, 2007). The n-3 to n-6 PUFA balance is more important than the absolute level of either fatty acid (Yehuda, Rabinovitz, & Mostofsky, 2008). An excess of LA inhibits its own desaturation, which could explain why a healthy body prefers a mediated balance over excessive levels of either n-3 or n-6 PUFA (Harzer, Dietrich, & Haug, 1984).

The n-3 to n-6 PUFA balance is important for homeostasis, normal growth, and development (Simopoulos, 2000). Therefore, finding the optimal ratio between n-3 and n-6 PUFA may be the most important objective for future research on the EFA. A 1:4 ratio of n-3 to n-6 PUFA was most effective in improving learning performance, elevating pain thresholds,

improving sleep, improving thermoregulation, and decreasing cholesterol levels in the membrane (Yehuda, Rabinovitz, & Mostofsky, 2008). However, Simopoulos (2000) suggested that the evolutionary ancestors to humans had a diet of an equal amount of both n-3 and n-6 PUFA, but through cultural, geographical, and technological evolution, humans have moved away from that consumption ratio to a variety of diverse diets across the world. The modern diet has detached itself from the evolutionary balance of n-3 and n-6 PUFA, and the reestablishment of the proper n-3 to n-6 PUFA ratio on a global scale could reduce some of the disease epidemics throughout humanity.

*PUFA consumption and the enhancement of cognition*

Of the vast amount of research focusing on n-3 PUFA, most studies have dealt with the effects of n-3 PUFA on pathologies or diseases rather than the benefits of n-3 PUFA on healthy individuals. Within the n-3 PUFA research specific to the CNS, researchers tend to focus on pathologies, such as Alzheimer's disease (Morris et al., 2003), age-related cognitive decline (Solfrizzi et al., 2006), stroke (He et al., 2002; Iso et al., 2001), and even Huntington disease (Puri et al., 2002). Thus, researchers have yet to effectively assess the benefits of n-3 PUFA on cognition in healthy populations, and the possible benefits could be significant.

Salvati et al. (2008) studied the effects of EPA on rat brains and found that EPA stimulated the expression of myelin-related proteins, which could explain the efficacy n-3 PUFA have on treating demyelinating diseases such as Alzheimer's disease (Boudrault, Bazinet, & Ma, 2009; Morris et al., 2003) and multiple sclerosis (Mehta, Dworkin, & Schwid, 2009). Additionally, if n-3 PUFA promote myelin growth throughout life, as they do during prenatal and perinatal development (Parra-Cabrera et al., 2007; Yehuda, Rabinovitz, & Mostofsky, 2008), they could ensure faster cognition and accordingly better cognitive function (Carlson, 2008;

Georgieff, 2007). Relating to the possible cognitive benefits of n-3 PUFA, Novak, Dyer, and Innis (2008) observed that feeding more DHA and less n-6 PUFA to piglets increased DHA concentrations in their frontal cortex when compared to piglets consuming a contemporary diet high in n-6 PUFA and low in n-3 PUFA. With a relationship between DHA and the frontal cortex, n-3 PUFA levels may relate to executive functions, such as inhibition or attention skills, explaining some of their efficacy in treating ADHD. As well, the EFA has a significant influence on the CNS (Yehuda, Rabinovitz, & Mostofsky, 2005), manifested in an array of physiological processes that could affect cognition. Multiple researchers have tied DHA to membrane fluidity and the neurotransmission of dopamine, acetylcholine, norepinephrine, and serotonin (Fontani et al., 2005; Gadoth, 2008; Kidd, 2007; Owen, Rees, & Parker, 2008). The psychophysiology of n-3 PUFA illustrates the potential of n-3 PUFA to direct changes in the brain, implying a probable effect on cognition.

*Rationale for the current investigation*. N-3 PUFA researchers have yet to devote a fair amount of attention to all possible populations that could benefit from n-3 PUFA in the diet. Most studies have focused on older populations or developing children. Only two studies have directly assessed n-3 PUFA consumption in relation to cognitive abilities among individuals of typical health. Kalmijn et al. (2004) assessed a middle-aged population between 45 and 70 years old, while Fontani et al. (2005) assessed a young adult population ranging from 22 to 51 years of age. Both found a positive relationship between n-3 PUFA in the diet and performances on cognitive tasks. Kalmijn et al. (2004) examined the effects of n-3 PUFA on cognition in a middle-aged population, finding an inverse relationship between reported n-3 PUFA consumption and impaired cognitive function and speed. Fontani et al. (2005) made a similar investigation among a younger population.

Studies on n-3 PUFA devoting their attention to young adult populations are rare (Fontani et al., 2005; Yehuda, Rabinovitz, & Mostofsky, 2005). Yehuda, Rabinovitz, and Mostofsky (2005) found that college students consuming a 1:4 n-3 to n-6 PUFA mixture experienced reduced cortisol levels alongside reduced test anxiety. In a population of healthy young adults, Fontani et al. (2005) found that n-3 PUFA supplementation led to increased vigor alongside reduced anxiety, aggression and depressive states. Additionally, Fontani et al. (2005) found a reduction of reaction time and better sustained attention among participants supplemented with n-3 PUFA. Few studies on n-3 PUFA have investigated a young adult and college-aged population, and the preliminary findings seem to indicate benefits on cognitive performance and brain health. These finding have led the current investigation to focus on the possible cognitive benefits of consuming foods high in n-3 PUFA on a college-aged population. We hypothesized that participants who frequently consume foods high in n-3 PUFA will perform better on tests of inhibition, attention, task-switching and short term memory (STM) than participants with little or no n-3 PUFA in their diet.

## Method

### *Participants*

Overall, 174 college students (N = 174) participated in this study, consisting of 122 females and 52 males with a mean age of 20.84 years (SD = 3.31). The entire pool of participants engaged in the first stage of the experiment. For the second stage of the experiment, the researcher called back 120 participants based on their self-reported n-3 PUFA consumption frequency. Only 64 participants returned to complete the second stage with one participant explaining that he was under the influence of alcohol upon arriving for testing. The researcher removed him from the experiment without penalty prior to testing. The participants that

completed the testing for the second stage of the experiment had 32 frequent n-3 PUFA consumers (n = 32) and 31 infrequent n-3 PUFA consumers (n = 31). The second stage participants had a mean age of 19.94 (SD = 1.54) with 49 being female and 13 being male. The researcher recruited participants from the Western Oregon University Human Participant Pool.

*Materials*

The study required few materials for the first stage of the experiment: an informed consent form (see Appendix A), a debriefing form (see Appendix B), and a food frequency questionnaire (FFQ) focused on weekly n-3 PUFA consumption (see Appendix C).

As various other studies have previously used an FFQ to assess n-3 PUFA consumption (Andersen, Solvoll, & Drevon, 1996; Hakkarainen et al., 2004; Helland et al., 2003; Howe et al., 2006; Kalmijn et al., 2004; Morris et al., 2003; Yamagishi et al., 2008), the researcher constructed a FFQ to identify the amount of fish, seafood, and other foods known to contain n-3 PUFA that participants regularly consumed. The FFQ assessed foods locally available to the participants. Each food item on the FFQ was readily available at a grocery store in close proximity to the research university in order to ensure that participants had some familiarity with the foods. The FFQ contained three domains of consumption: seafood, non-seafood high in n-3 PUFA, and supplementation.

Due to the wide variety of studies that have specifically assessed consumption rates of seafood for its high concentrations of DHA and EPA, seafood served as its own category, as seafood consumption accounts for a high rate of n-3 PUFA consumption (Bulliyya, 2002; Crawford, 1992; Daniels et al., 2004; Dewailly et al., 2003; Gebauer et al., 2006; Hakkarainen et al., 2004; Oomen et al., 2000). Within the non-seafood domain, a variety of foods known to contain n-3 PUFA were represented, further categorized into two subdomains characterized by

the estimated serving sizes of each food. The subdomains included a main portion size grouping and a supplemental food grouping. The main portion size subdomain included various items that researchers suggest contain significant levels of n-3 PUFA, specifically grass-fed beef (Howe et al., 2006; Ponnampalam, Mann, & Sinclair, 2006; Winningham, 2009), n-3 fortified eggs (Kidd, 2007; Ohman et al., 2008; Shapira, Weill, & Loewenbach, 2008; Winningham, 2009), and lamb meat (Howe et al., 2006). The supplemental food subdomain included walnuts (Gebauer et al., 2006; Gillen et al., 2005; Winningham, 2009) and flaxseed (Gebauer et al., 2006; Winningham, 2009), both botanical foods known for their high concentrations of n-3 PUFA.

The third domain of supplementation included fish oil and flaxseed oil supplements of 1000 mg or higher, a value derived from the American Heart Association which recommends 1000 mg of DHA/EPA per day (Kidd, 2007). Many researchers have examined fish oil supplementation to assess the various effects of n-3 PUFA (Amminger et al., 2007; Barceló-Coblijn et al., 2008; Carlson et al., 1992; Fortin et al., 1995; Helland et al., 2003; Novak, Dyer, & Innis, 2008; Olafsdottir et al., 2006; Riediger et al., 2008; Simopoulos, 2001; Simopoulos, 2003) and some literature has also observed a similar efficacy in flaxseed oil supplementation (Barceló-Coblijn et al., 2008; Riediger et al., 2008).

The FFQ contained questions regarding the frequency of consumption per week, as studies such as Morris et al. (2003) demonstrated observable benefits of n-3 PUFA through fish consumption at least once a week, while other studies – such as Daniels et al. (2004) – observed participants consuming fish four times a week and perceived benefits in association with that rate of seafood consumption. In the current study, the researcher assessed food consumption frequency alongside perceived portion size and n-3 PUFA levels to estimate the amount of n-3 PUFA consumed weekly by each participant.

To measure n-3 PUFA consumption levels from the responses to the FFQ, the researcher developed point values for each food to estimate the amount of n-3 PUFA in the weekly diet. The researcher then multiplied the point value of each food by the amount of days the participant consumed the food per week to assess the total n-3 PUFA in the diet from that particular food. Thereafter, the researcher added the n-3 PUFA consumption ratings from each food, compiling a total n-3 PUFA consumption score from all foods on a scale from 0 to 287.

Each food on the FFQ received a different point value determined by the researcher after examining previous research on foods rich in n-3 PUFA. Due to the array of studies focusing on DHA (Bucher et al., 2002; Clandinin et al., 1989; Crawford, Hassam, & Williams, 1976; Helland et al., 2003; Innis, 2003; Novak, Dyer, & Innis, 2008; Oomen et al., 2000; Ruxton, 2007; Smit et al., 2002; Yehuda, Rabinovitz, & Mostofsky, 1999; Yehuda, Rabinovitz, & Mostofsky, 2008), fish containing significant levels of DHA (Oomen et al., 2000; Ruxton, 2007; Simopoulos, 2000), and a variety of studies observing benefits of n-3 PUFA through marine diets (Bulliyya, 2002; Crawford, 1992; Dewailly et al., 2003; Kalmijn et al., 2004), seafood received the highest point value on the FFQ with 10 points. Fish oil supplementation had the second highest point value with 8, as Novak, Dyer, and Innis (2008) concluded that supplementation increased DHA and a variety of studies used fish oil supplementation and observed clear results (Barceló-Coblijn et al., 2008; Carlson et al., 1992; Fortin et al., 1995; Helland et al., 2003; Novak, Dyer, & Innis et al., 2008; Olafsdottir et al., 2006; Riediger et al., 2008). The third highest point value went to n-3 fortified eggs, which an array of literature supports as a significant source of n-3 PUFA (Kidd, 2007; Ohman et al., 2008; Shapira, Weill, & Loewenbach, 2008; Winningham, 2009). A serving of three to four eggs received 6 points, while one to two eggs received 3 points. Next, lamb and grass-fed beef received the same point value of 4, as Howe et al. (2006) suggested that

they had an equal n-3 PUFA contribution through the diet. However, grass-fed beef was not included in the final scoring of the FFQ. Participants frequently reported a misunderstanding as to whether or not their beef was grass-fed, leading to a substantial amount of error in reporting.

Lastly, botanical sources – flaxseed, flaxseed oil, and walnuts – received a point value of 2. Despite some research observing similar results between fish oil and flaxseed oil (Barceló-Coblijn et al., 2008; Riediger et al., 2008), other research has observed it as ineffective in increasing DHA (Francois, Connor, Bolewicz, & Connor, 2003), and Kidd (2007) along with Larsson, Kumlin, Ingelman-Sundberg, and Wolk (2004) suggest that flaxseed oil cannot necessarily substitute for other sources of DHA and EPA in the diet. Walnuts were categorized with flaxseed as a botanical source of n-3 PUFA, as previous research (Griel et al., 2007) grouped walnuts with flaxseed oil as a source of ALA, but not DHA, and the current researcher could find no research defending walnuts as a significant source of DHA in the diet.

The FFQ also included a section of inquiries into demographic and contact information, but after compiling all demographic data in association with the food frequency ratings, the researcher removed and destroyed the demographic section of the FFQ, disassociating participants' identities with their respective responses. Lastly, the debriefing form explained the purpose of the first stage of the study to participants, and explained that the researcher may contact them for a follow-up series of tests.

The second stage of the experiment required more materials including a second informed consent form (see Appendix D). Participants completed a Digit-span Test, which required participants to recall a series of numbers in order to assess their STM capacity (Geva, Eshel, Leitner, Fattal-Valevski, & Harel, 2008; Koppitz, 1981; Miller, 1956). The researcher also incorporated a Stroop Test to examine the participants' inhibition and attention (Coderre, Filippi,

Newhouse, & Dumas, 2008; MacLeod, 1991; Stroop, 1935). Lastly, participants completed a Trail Making Test (TMT) to measure attention and task-switching (Corrigan & Hinkeldey, 1987; Lezak, 1995; Reitan, 1958). After this stage of the experiment, the participants received a second debriefing form explaining the overarching purpose of both stages of the study (see Appendix E). After the researcher collected and analyzed all the data, he sent a final debriefing form via email (see Appendix F) to all participants explaining the purpose, hypothesis, and results of the study.

*Procedure*

*Stage one*. The researcher met with participants either singularly or in large groups of 20 to 60 participants. Participants read and signed the first informed consent form. Next, the researcher passed out the FFQ, questioning participants about demographic and dietary information. After the participants completed the FFQ, the researcher collected them and distributed the first debriefing forms to all of the participants.

*Stage two*. After compiling the data, the researcher examined the FFQ responses of participants consuming the most and least n-3 PUFA weekly. The researcher performed an approximate tertiary split and called back 120 participants altogether, with 60 being the most frequent n-3 PUFA consumers and the other 60 being the most infrequent n-3 PUFA consumers. The researcher contacted these participants via email, and met with the participants that opted to participate in the second stage of the experiment. Altogether, 63 participants returned, with 32 frequent n-3 PUFA consumers (n = 32) and 31 infrequent n-3 PUFA consumers (n = 31). The researcher administered a series of cognitive tests on the participants in the second stage, and took measurements on participants' performances as they completed three tests in the following order: a digit-span test, a Stroop Test, and a TMT. Once the participants completed all required tests, the researcher gave the participants the second debriefing form.

After the researcher completed data collection and analysis, he contacted all participants via email with a final debriefing form that included the results of the experiment.

## Results

### *Descriptive Statistics on n-3 PUFA Consumption amongst Participants*

In the first stage of the experiment, all participants (N = 174) completed the FFQ and reported their weekly consumptions of foods known to contain n-3 PUFA. See Table 1 for the average weekly consumption of each n-3 PUFA food group on the FFQ and the average weekly n-3 PUFA score on the FFQ across all participants. For both the low consumer group in the second stage of the experiment (n = 31) and the high consumer group in the second stage of the experiment (n = 32), see Table 1 for their average weekly consumption of each n-3 PUFA food group on the FFQ and the average weekly n-3 PUFA score on the FFQ.

### *The Effects of n-3 PUFA Consumption on Digit-Span Test Performance*

When administering the digit-span test, measurement began at a series of 4 digits for participants to recall. Each participant received two opportunities at recalling as many digits as possible, and an average of the two attempts was taken to provide them with a score. If a participant failed to recall the digits on their attempt at the initial four digit sequence, that measurement was voided and the participant's other attempt was taken as their score.

An independent samples t-test was performed to assess the effects of reported n-3 PUFA consumption on average length of digit span, measured as the number of digits remembered (more digits was indicative of better performance and greater STM). The t-test indicated that there was not a reliable difference in mean digit span as a function of n-3 PUFA group, $t(61) = 1.08$, $p = .29$. The mean digit span was 6.18 ($SD = 1.01$) digits for the lower consumers and 5.89

(*SD* = 1.11) digits for the higher consumers. See Figure 1 to view means for STM as a function of consumption group.

*The Effects of n-3 PUFA Consumption on the Stroop Test Performance*

When administering the Stroop Test, an error occurred in data collection, resulting in the removal of some data from analysis. Altogether, the Stroop Test scores of 11 participants were not valid scores, resulting in smaller low consumer (n=25) and high consumer groups (n=27).

An independent samples t-test was performed to assess the effects of reported n-3 PUFA consumption on time to complete the incongruent Stroop Test (the word and the color of the text were incongruent), measured in seconds (quicker completion was indicative of better performance). The t-test indicated that there was not a reliable difference in mean completion times as a function of n-3 PUFA group, $t(50) = 0.21$, $p = 0.83$. The mean time to complete the incongruent Stroop Test was 41.80 (*SD* = 9.19) seconds for the lower consumers and 42.33 (*SD* = 6.66) seconds for the higher consumers. See Figure 2 to view means of incongruent Stoop Test performances as a function of consumption group.

A 2 X 2 mixed subjects ANOVA indicated no interaction between consumption group and Stroop type (congruent or incongruent), $F(1, 50) = 0.63$, $p = .43$. The statistical analysis indicated a main effect for Stroop type, $F(1, 50) = 303.65$, $p < .001$, but no main effect for consumption group, $F(1, 50) = 1.13$, $p = .29$.

*The Effects of n-3 PUFA Consumption on the TMT*

An independent samples t-test was performed to assess the effects of reported n-3 PUFA consumption on time to complete part B of the TMT, measured in seconds (quicker completion was indicative of better performance). The t-test indicated that there was not a reliable differences in mean completion times of TMT part B as a function of n-3 PUFA group, $t(61) =$

1.35, $p = .18$. The mean time to complete TMT part B was 39.64 ($SD = 15.35$) seconds for the lower consumers and 45.17 ($SD = 16.95$) seconds for the higher consumers. See Figure 2 to view means of TMT part B performance as a function of consumption group.

A 2 X 2 mixed subjects ANOVA indicated no interaction between consumption group and part of the TMT (parts A and B), $F$ (1, 61) = 2.00, $p = .16$. The statistical analysis indicated a main effect for TMT part, $F$ (1, 61) = 171.34, $p < .001$, but no main effect for consumption group, $F$ (1, 61) = 1.42, $p = .24$.

## Discussion

When comparing high n-3 PUFA consumers to low n-3 PUFA consumers, the results demonstrated no significant relationship between the performances on any cognitive test and the reported amount of n-3 PUFA consumed on a weekly basis. When compared to the high n-3 PUFA consumers, the low n-3 PUFA consumers actually had a higher STM on average and performed better on tasks of inhibition, attention, and task-switching. The differences between groups were not significant, but run contrary to previous findings assessing n-3 PUFA and cognition. These results oppose the preliminary findings of other researchers observing the benefits of n-3 PUFA on cognitive tasks among similar populations (Fontani et al., 2005; Kalmijn et al., 2004). Though disparate results are typical across n-3 PUFA studies (Church et al., 2009; Gadoth, 2008; Hakkarainen et al., 2004; Meyer, et al., 2001; Politi et al., 2008; Yamagishi et al., 2008), confounding variables may have contributed to these counterintuitive results.

When considering performance on the cognitive tests, the researcher did not control for many confounding variables. Though each confounding variable had an equal likelihood of occurring in either group, they may have contributed to errors in cognitive performance. Caffeine

notably provides individuals with a cognitive advantage when administered in an appropriate dosage (Killgore, Kahn-Greene, Grugle, Killgore, & Balkin, 2009; Smith, Brice, Nash, Rich, & Nutt, 2003). One participant underwent the cognitive tasks while drinking from a coffee thermos, while other participants may have consumed caffeine as well. Many other participants could have been under the influence of other drugs with effects on cognition. One participant implied that he consumed alcohol prior to the study and his cognitive performance was not observed or analyzed. Alcohol has notable detrimental effects on cognitive performance (Hindmarch, Kerr, & Sherwood, 1991), and other participants could have consumed alcohol prior to the testing, unbeknownst to the researcher. These cofounding uncontrolled effects on cognition may have construed the cognitive performances of participants in either group, giving certain participants either advantages or disadvantages in cognitive performance.

Aside from pharmacological variables, variables of stress and anxiety could have hurt the cognitive performances of participants. The study took place partially during the final week of examinations in the academic term of the research university. Accordingly, fatigue and sleep deprivation may have adversely affected cognitive performances. Falleti, Maruff, Collie, Darby, and McStephen (2003) demonstrated how 24 hours of wakefulness had comparable if not greater detriments on cognition to that of alcohol. One participant explained that she suffered from a sleep disorder and had irregular sleeping habits. Such detriments on cognition went uncontrolled across both conditions.

Additionally, considering the timeframe of the experiment, test anxiety may have contributed to stress, which could have inadvertently depreciated or enhanced cognitive performances. Yehuda, Rabinovitz, and Mostofsky (2005) assessed test anxiety in relation to the EFA, examining the prevalence of stress via cortisol levels. Cortisol is a glucocorticoid, which is

a group of hormones of the adrenal cortex that secrete most commonly in times of stress (Carlson, 2008). When performing cognitive tasks, the influence of stress has historically had some range of influence. Houston (1969) found that participants in an aroused state performed worse on the Stroop Test than individuals not aroused. Contrarily, Chajut and Algom (2003) observed improvements in selective attention when under stressful conditions. As well, Saville (2009) observed an association between better inhibitory abilities and higher cortisol responsiveness. Therefore, test anxiety and stress could have had an array of effects on cognitive performance throughout the experiment.

Aside from confounding variables affecting cognitive performances, problems may have derived from participants' self-reporting of their diet. When self-reporting their weekly dietary consumption, participants may have inaccurately reported their food consumption, which in turn led to inaccurate n-3 PUFA scores. The responses for weekly consumption for grass-fed beef were not scored, as multiple participants vocalized their uncertainty with the researcher, not knowing whether or not their beef was grass-fed. The FFQ only allowed for a numerical response of times per week that participants consumed certain foods. Therefore, when administered to large groups of participants at one time, individual participants that misunderstood certain questions could not vocalize their confusion and may have responded inaccurately.

One participant reported consuming 7 servings of 1 to 2 n-3 PUFA fortified eggs each week. The same participant reported consuming 7 additional servings of 3 to 4 n-3 PUFA fortified eggs each week. Therefore, this participant reported consuming between 28 and 42 n-3 PUFA fortified eggs each week, a highly unusual diet for a normal individual. Following this example of odd reporting, the FFQ may not have misled participants, but instead participants

may have not known their actual consumption frequencies for certain foods. Accordingly, some participants provided outlandish rates of consumption for certain foods, demonstrating that they may have reported inaccurately on the FFQ.

Additionally, the FFQ could have asked for too broad or too specific of a time frame in order to accurately assess n-3 PUFA consumption. When assessing some food groups, participants may not have consumed them on a weekly basis, and therefore, the participants may not have accurately assessed their frequency of consumption for these foods. If the participant consumed fish every other week, they may have reported no fish consumption, but if they reported that they consumed fish once a week, they would have reported twice their actual consumption. Therefore, someone categorized as a high n-3 PUFA consumer may have an inaccurately reported consumption rating. Without a methodology for measuring plasma concentrations of n-3 PUFA among participants, the researcher had to trust the reported diets of participants, regardless of whether those reports accurately represented their n-3 PUFA intake and absorption. The timeframe for reported consumption may have worked more effectively if expanded to a month. He et al. (2002) observed a reduced likelihood of ischemic strokes among male individuals consuming fish just once to thrice a month. Though the relationship between n-3 PUFA and likelihood of stroke may have no relationship with overall cognition, He et al. (2002) does demonstrate how even a small amount of consumption may have an effect on the brain.

Another problem with reporting may concern how the FFQ emphasized a variety of food groups known to contain n-3 PUFA. Many other studies focused primarily on one food, such as Gillen et al. (2005) focusing primarily on walnut consumption, while Barceló-Coblijn et al. (2008) focused primarily on fish oil and flaxseed oil. By including a variety of food groups, the

FFQ may have emphasized less significant n-3 PUFA sources over more significant n-3 PUFA sources. The high n-3 PUFA consumers averaged a weekly n-3 PUFA score of 46.22, which equals an n-3 PUFA rating of about four and a half servings of fish on the FFQ scoring scale. However, the high consumers only averaged a fish consumption rate of 1.88 servings per week. The majority of studies have focused on marine sources of n-3 PUFA (Bulliyya, 2002; Crawford, 1992; Dewailly et al., 2003) and fish oil sources of n-3 PUFA (Barceló-Coblijn et al., 2008; Carlson et al., 1992; Fortin et al., 1995; Helland et al., 2003; Novak, Dyer, & Innis et al., 2008; Olafsdottir et al., 2006; Riediger et al., 2008). Even Kalmijn et al. (2004) emphasized that fatty fish and marine n-3 PUFA consumption aligned with a reduced risk of cognitive impairment. Marine foods and oils may have required more emphasis on the FFQ, with the current scoring structure rewarding alternative n-3 PUFA sources perhaps too greatly. Without that emphasis on marine sources of n-3 PUFA, participants consuming alternative n-3 PUFA sources may have been categorized in the high n-3 PUFA consumer group despite consuming low amounts of marine n-3 PUFA at best.

Alternatively, the FFQ may have emphasized marine sources too much. With marine sources containing high amounts of DHA (Oomen et al., 2000; Ruxton, 2007; Simopoulos, 2000), the FFQ provided marine foods with the highest possible n-3 PUFA score. However, Salvati et al. (2008) emphasized the importance of EPA in myelinogenesis. The FFQ emphasized the high levels of DHA and EPA in fatty fish (Oomen et al., 2000), but did not assess the consumption levels of DHA or EPA across food groups. Marine sources contain both, but in supplementation studies, the levels of both types of n-3 PUFA may vary depending on the source and the dosage (Amminger et al., 2007; Barceló-Coblijn et al., 2008; Carlson et al., 1992; Fortin et al., 1995; Helland et al., 2003; Novak, Dyer, & Innis, 2008; Olafsdottir et al., 2006; Riediger

et al., 2008; Simopoulos, 2001; Simopoulos, 2003). Therefore, participants may not have consumed very much EPA in their diet, and therefore did not observe the cognitive benefits.

Another perspective of the results may interpret them as valid and preliminary. The population served as a newly tested age group. Fontani et al. (2005) tested the cognitive effects of n-3 PUFA on an age group between 22 and 51 years of age, while Yehuda, Rabinovitz, & Mostofsky (2005) stand as the only previous researchers observing the anxiolytic effects of n-3 PUFA on a college population. The population in the current study had a mean age of 20.84 years (SD = 3.31). This age group of late adolescence and emerging adulthood has yet to be assessed and n-3 PUFA may have no direct effect on their cognition. Muthayya et al. (2009) examined the effects of dietary n-3 PUFA on a population of children in developing areas of India. They administered high and low concentrations of n-3 PUFA to different experimental groups in the population, attempting to observe a difference that the concentration of n-3 PUFA had on cognitive performance. Muthayya et al. (2009) found no significant difference in cognitive performances between high and low concentrations of n-3 PUFA in the diet. Similarly, the results in the college population of the current study observed no difference between high and low n-3 PUFA consumers. Perhaps the cognitive effects of n-3 PUFA appear during later age, as n-3 PUFA consumption does protect against cognitive impairment into late adulthood (Eskelinen et al., 2008; Morris et al., 2003; Solfrizzi et al., 2006).

However, Fontani et al. (2005) emphasized neurotransmitters and pathways possibly affected by n-3 PUFA, such as dopamine, acetylcholine, norepinephrine, and serotonin. Studies observing the amelioration of aggression via n-3 PUFA consumption also support the influence of n-3 PUFA on serotonergic neurotransmission (Bell et al., 2004; Itomura et al., 2005). Therefore, just as Fontani et al. (2005) observed benefits on attention, n-3 PUFA consumption

should have some effect on neurocognition due mostly to its importance in the CNS (Gadoth, 2008; Hallahan & Garland, 2005; Kidd, 2007; Owen, Rees, & Parker, 2008). However, these effects may not present themselves until later age, despite n-3 PUFA consumption being increasingly important throughout the lifespan. The accretion of the EFA increases significantly during the final trimester of pregnancy (Clandinin et al., 1980) and fetal concentrations of DHA depends significantly on the maternal diet (Innis, 2003). The role of PUFA in prenatal development computes to greater cognitive performance at later age, as demonstrated in higher IQ scores and better cognitive abilities during childhood among children of high n-3 PUFA consuming mothers (Helland et al., 2003; Innis, 2003; Koletzko et al., 2001; Larque, Demmelmair, & Koletzko, 2002). The benefits of n-3 PUFA in late adolescence and emerging adulthood may exist latently, only exhibiting their benefits through their preventative qualities of diseases and cognitive deficits later in life (Dewailly et al., 2003; Eskelinen et al., 2008; Iso et al., 2001; Kagawa et al., 1982; Ruxton, 2007; Simopoulos, 2001).

Another disheartening result of the experiment came with the population overall consuming low levels of marine n-3 PUFA across the population. Kalmijn et al. (2004) emphasized the importance of marine n-3 PUFA for cognitive effects; however, as shown in Table 1, the marine n-3 PUFA scores were overall low, with the mean weekly seafood consumption for high consumers being just 1.88 servings of seafood per week and the mean weekly fish oil supplementation for high consumers being just 1.34 capsules of 1000 mg or more n-3 PUFA per week. Some studies observing the physiological benefits of n-3 PUFA administered fish oil supplementation on a daily basis (Amminger et al., 2007; Bucher et al., 2002; Helland et al., 2003). Even the American Heart Association suggests 1000 mg DHA/EPA per day (Kidd, 2007). Very few researchers have investigated the influence of n-3 PUFA on

cognition in healthy or youthful populations (Fontani et al., 2005; Kalmijn et al., 2004; Muthayya et al., 2009; Yehuda, Rabinovitz, & Mostofsky, 2005), and accordingly researchers have yet to determine an optimal dosage, source, or frequency of consumption of n-3 PUFA in order to obtain cognitive benefits.

Future research must find participants with higher rates of n-3 PUFA consumption in order to identify a possible effect. Controlling the dietary intake of n-3 PUFA could better control participants misreporting their diet on the FFQ. As well, observing plasma concentration of n-3 PUFA would provide more direct physiological evidence when relating n-3 PUFA to some behavioral effect. Though misreporting may have occurred, the data collected from the FFQ had overall disheartening features to it. Considering the overall consumption rates across the population, the data gathered demonstrates how this specific college population consumes very low amounts of food containing n-3 PUFA and more specifically low amounts of marine n-3 PUFA. Across the entire population, n-3 PUFA consumption was staggeringly low, with a mean n-3 PUFA total score of 23.06. This score equates to approximately two servings of seafood a week on the scoring scale for the FFQ. Still, over half of these points on average did not come from marine n-3 PUFA sources, as the mean seafood consumption rating across the population was just 0.94 servings of seafood a week. Generally heavily marine diets within populations lead to significant reductions in the prevalence of diseases and cognitive deficits as the populations mature (Dewailly et al., 2003; Eskelinen et al., 2008; Iso et al., 2001; Kagawa et al., 1982; Ruxton, 2007; Simopoulos, 2001). Considering the importance of n-3 PUFA in preventing and managing certain pathologies and diseases, the low n-3 PUFA consumption across a college population may compute to a higher prevalence of diseases and cognitive deficits in later age.

A great concern regarding n-3 PUFA deals with its overall disappearance from the modern human diet (Innis & Jacobson, 2007; Simopoulos, 2000; Simopoulos, 2006), and the data gathered from the FFQ further supports this conclusion. Without a large amount of n-3 PUFA in the diet, humans warp their n-3 to n-6 PUFA ratio, which may translate to a negative effect on their health. As humans have no enzymatic mechanism to interconvert the EFA (Simopoulos, 2006), they must rely on collecting the correct nutrients from their diet to balance this ratio. Both EFA are required in the human diet (Crawford, 1992). If the ratio between the two PUFA becomes too large, it affects both the absorption of the nutrients and the overall health of the individual. According to Novak, Dyer, and Innis (2008), the EFA compete for absorption leading to increased n-6 PUFA absorption alongside decreased DHA absorption. With DHA being critical for the CNS (Gadoth, 2008; Kidd, 2007; Owen, Rees, & Parker, 2008), any opposition to its absorption could present some hazard to normal physiological function. Ultimately, the balance between the EFA appears more important than the absolute level of either (Yehuda, Rabinovitz, & Mostofsky, 2008) and the low n-3 PUFA consumption rate of the college population observed through the FFQ may suggest a large n-3 to n-6 PUFA ratio.

When assessing a college population, Yehuda, Rabinovitz, & Mostofsky (2005) administered participants with a mixture of the EFA composed of a low n-3 to n-6 PUFA ratio of 1:4. Other studies have supported this ratio as producing positive physiological effects (Simopoulos, 2006; Yehuda, Rabinovitz, & Mostofsky, 2008). With this low ratio producing an anxiolytic effect in a college population, perhaps the college population requires this low ratio to acquire cognitive benefits as well. Other studies have supported this ratio as producing positive physiological changes in other aspects of health (Simopoulos, 2006; Yehuda, Rabinovitz, & Mostofsky, 2008). The FFQ did not account for the consumption of n-6 PUFA and could not

effectively assess an n-3 to n-6 PUFA ratio in the overall diet of the population; however, with the modern dietary trend consuming higher amounts of n-6 PUFA (Innis & Jacobson, 2007; Simopoulos, 2000; Simopoulos, 2006), this population likely has a similarly large ratio.

The suggested dietary intakes of n-3 PUFA vary between advisory bodies (American Diabetes Association, 2008; Kidd, 2007; Ruxton, 2007); however, the low n-3 to n-6 PUFA ratio seems most important (Yehuda, Rabinovitz, & Mostofsky, 2008). Therefore, n-3 PUFA require a reintroduction into the human diet. In school children and college students, administrative bodies have a greater opportunity to control the diet, by making n-3 PUFA available and accessible to all students. As demonstrated by the data from the FFQ, the population of college students consumes a low amount of n-3 PUFA and may consume high levels of n-6 PUFA. However, researchers have demonstrated the preventative qualities of n-3 PUFA in multiple diseases, emphasizing their importance in the diet throughout the lifespan. Researchers have observed benefits to n-3 PUFA in the treatment and prevention of five of America's top ten causes of death (Kung et al., 2008): cardiovascular disease (Bucher et al., 2002; Bulliyya, 2002; Crawford, 1992; Dewailly et al., 2003; Yamagishi et al., 2008), cancer (Donaldson, 2004; Ge et al., 2002), stroke (He et al., 2002; Iso et al., 2001), diabetes mellitus (Gillen et al., 2005; Kagawa et al., 1982; Nettleton & Katz, 2005) and Alzheimer's disease (Boudrault, Bazinet, & Ma, 2009; Morris et al., 2003). Altogether, these five afflictions and diseases caused 1.5 million deaths in 2005 (Kung et al., 2008). With the significant toll of these diseases on society – both emotionally and economically – n-3 PUFA may offer a conventional and affordable method of preventative health care and possible treatment. Though cognitive benefits may not appear within this college population, n-3 PUFA remain an essential nutrient and the ratio between the EFA remains of the utmost importance for human health.

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Table 1

*Mean N-3 PUFA Weekly Consumption Rate Scores from FFQ and Mean Weekly Food Group Consumption Rates*

| | Omega-3 Total FFQ Score | Seafood | Omega-3 Eggs Serving of 1-2 | Omega-3 Eggs Serving of 3-4 | Lamb | Flaxseed | Walnut | Flaxseed Oil (1000mg or more) | Fish Oil (1000mg or more) |
|---|---|---|---|---|---|---|---|---|---|
| All Participants (N = 174) | 23.06 (SD = 23.49) | 0.94 (SD =1.13) | 1.5 (SD = 1.63) | 0.51 (SD = 1.20) | 0.02 (SD = 0.15) | 0.68 (SD = 1.38) | 0.58 (SD = 1.20) | 0.20 (SD = 0.87) | 0.41 (SD = 1.43) |
| Low Consumer Group (n = 31) | 4.23 (SD = 3.75) | 0.13 (SD = 0.34) | 0.48 (SD = 0.81) | 0.06 (SD = 0.25) | 0 (SD = 0) | 0.32 (SD = 0.70) | 0.23 (SD = 0.50) | 0 (SD = 0) | 0 (SD = 0) |
| High Consumer Group (n = 32) | 46.22 (SD = 21.96) | 1.88 (SD = 1.52) | 2.09 (SD = 1.42) | 0.81 (SD = 1.23) | 0.06 (SD = 0.25) | 1.25 (SD = 1.74) | 1 (SD = 0.50) | 0.34 (SD = 1.29) | 1.34 (SD = 2.67) |

Figure Caption

*Figure 1*. Mean participant performance on digit span test for both high and low n-3 PUFA consuming groups.

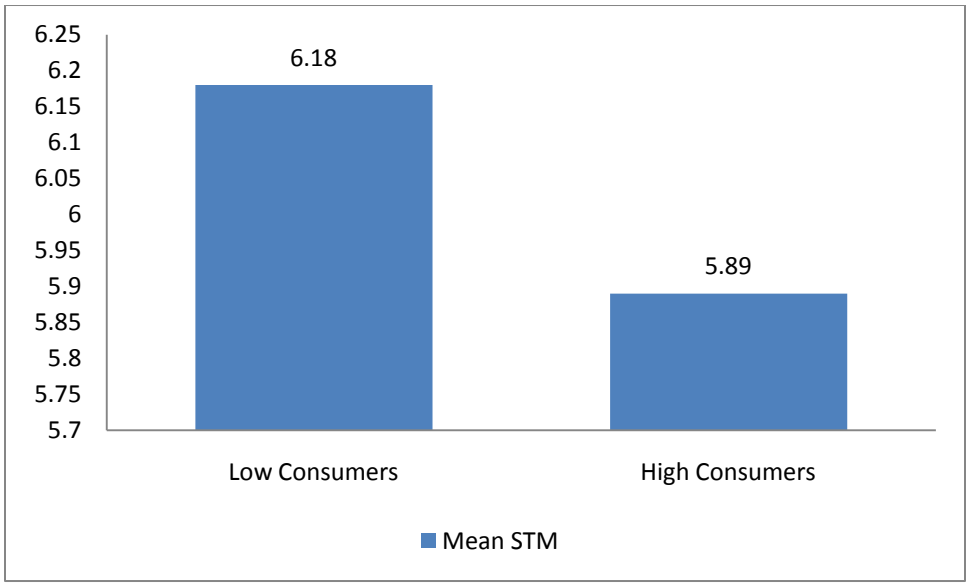
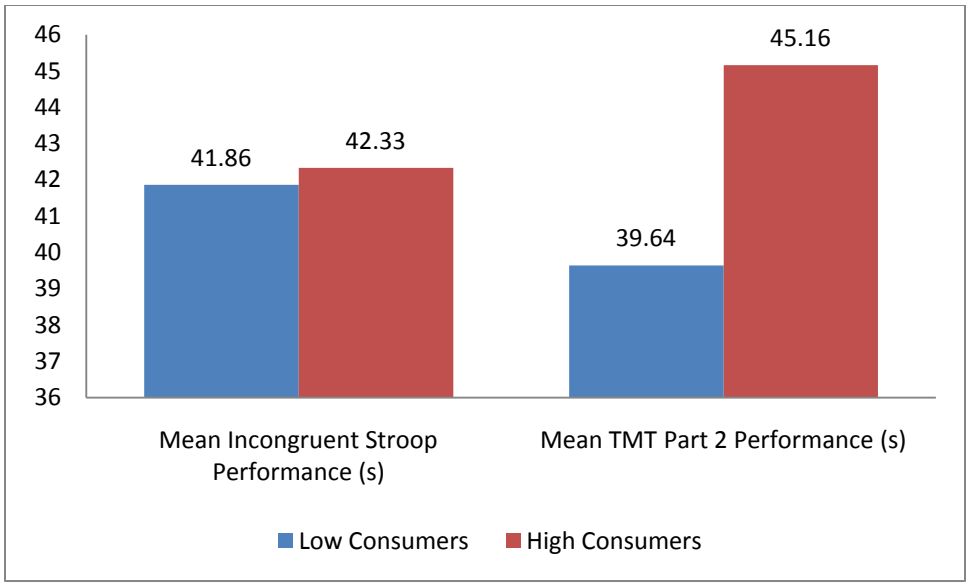

6.25
6.2
6.15
6.1
6.05
6
5.95
5.9
5.85
5.8
5.75
5.7
6.18
5.89
Low Consumers
High Consumers
Mean STM

Figure Caption

*Figure 2*. Mean participant performance on incongruent Stroop and TMT part 2 in seconds for both high and low n-3 PUFA consuming groups.


46
45
44
43
42
41
40
39
38
37
36
41.86
42.33
39.64
45.16
Mean Incongruent Stroop Performance (s)
Mean TMT Part 2 Performance (s)
Low Consumers
High Consumers

# Appendix A

## Omega-3 Polyunsaturated Fatty Acids and their Effects on Cognition

Western Oregon University and the Department of Psychology support the practice of protecting the rights of research participants. Accordingly, this project has been reviewed and approved by the WOU Institutional Review Board. The information in this consent form is provided so that you can decide whether you wish to participate in this study. It is important that you understand that your participation is considered voluntary. You may elect, either now or at any time during the experiment, to withdraw your participation, with no penalty or loss of benefits.

This study is an investigation of food consumption and its effects on cognition. You will be asked to complete a survey to earn extra credit, and depending on the results of your survey, you may be contacted with an opportunity for more extra credit through participation in a series of cognitive tests.

Your response will only be viewed by project personnel and they will be kept in a locked file cabinet in my office until January 2012 when they will be destroyed. During this period only the principal investigators will have access to the responses.

This experiment poses no known risk to your health. Once all of the data has been collected, we will remove information that can connect your identity with the information you report. For participation in this research project, you will receive extra credit at the conclusion of each stage of the study. After completing the first stage, the researcher will grant you extra credit, but depending on the data you provide in the first stage of the experiment, the researcher may contact you requesting your further participation in the experiment, through which you could receive more extra credit. Also, upon completion of your participation in this study you will be provided with a brief explanation of the question this study addresses. If you have any questions not addressed by this consent form, please do not hesitate to ask. You will receive a copy of this form, which you should keep for your records. We thank you for your time.

Justin Karr
jkarr06@wou.edu

Dr. Rob Winningham
Professor of Psychology
Todd 322, 503-838-8316
winninr@wou.edu

CONSENT STATEMENT:
I have read the above comments and agree to participate in this experiment. I understand that if I have any questions or concerns regarding this project I can contact the principal investigator at the above location or the WOU Institutional Review Board at (503) 838-8271.

_____

Signature Date

# Appendix B

## Debriefing Form

Thank you for your participation in the first stage of this study. The purpose of this stage of the study was to assess participants' frequency in consuming foods containing omega-3 polyunsaturated fatty acids. Some participants that consume certain amounts of the foods listed on the survey will be contacted by the researcher with an opportunity to participate in a second stage of the research, where more extra credit could potentially be granted.

In the conclusion of the experiment, all participants of the study will receive a debriefing email explaining in detail the purposes and results of the study.

If this study induced any degree of unnecessary stress, or if you feel any degree of discomfort after your participation in this study, please do not hesitate to contact the WOU Counseling Center at 503-838-8396.

If you have any further questions please contact the researcher Justin Karr at jkarr06@wou.edu.

Appendix C

Full Name:_____ Age:_____ Sex: M F Email:_____

_____ _____ _____ _____ _____ _____ _____

Please answer all of the following questions to the best of your ability. If you do not know an exact value, estimates and averages will work.

How many days of the week do you consume fish or seafood products (ex. Salmon, Crab, Fish Sticks, Anchovies, Sushi, Shellfish, etc.)?

0 1 2 3 4 5 6 7

How many days of the week do you consume beef that you know to be grass-fed?

0 1 2 3 4 5 6 7

How many days of the week do you consume a serving of one to two eggs that you know were produced by grass-fed chickens and/or contain high levels of omega-3 fatty acids?

0 1 2 3 4 5 6 7

How many days of the week do you consume a serving of three or more eggs that you know were produced by grass-fed chickens and/or contain high levels of omega-3 fatty acids?

0 1 2 3 4 5 6 7

How many days of the week do you consume lamb meat?

0 1 2 3 4 5 6 7

How many days of the week do you consume flaxseed or a product you know contains flaxseed?

0 1 2 3 4 5 6 7

How many days of the week do you consume walnuts?

0 1 2 3 4 5 6 7

How many days of the week do you consume flaxseed oil supplement of 1000mg or more?

0 1 2 3 4 5 6 7

How many days of the week do you consume fish oil supplement of 1000 mg or more?

0 1 2 3 4 5 6 7

# Appendix D

## Omega-3 Polyunsaturated Fatty Acids and their Effects on Cognition

Western Oregon University and the Department of Psychology support the practice of protecting the rights of research participants. Accordingly, this project has been reviewed and approved by the WOU Institutional Review Board. The information in this consent form is provided so that you can decide whether you wish to participate in this study. It is important that you understand that your participation is considered voluntary. You may elect, either now or at any time during the experiment, to withdraw your participation, with no penalty or loss of benefits.

This study is an investigation of food consumption rate and their effects on cognition. You have previously completed a survey regarding your dietary habits, and due to the results of your survey, you have been contacted once again with an opportunity for more extra credit through participation in a series of cognitive tests.

Your response will only be viewed by project personnel and they will be kept in a locked file cabinet in my office until January 2012 when they will be destroyed. During this period only the principal investigators will have access to the responses.

This experiment poses no known risk to your health. Once all of the data has been collected, we will remove information that can connect your identity with the information you report. For participation in this stage of the research, you will receive extra credit at the conclusion of a series of cognitive tests. Upon completion of your participation in this stage of the study you will be provided with a brief explanation of the question this study addresses. If you have any questions not addressed by this consent form, please do not hesitate to ask. You will receive a copy of this form, which you should keep for your records. We thank you for your time.

Justin Karr
jkarr06@wou.edu

Dr. Rob Winningham
Professor of Psychology
Todd 322, 503-838-8316
winninr@wou.edu

CONSENT STATEMENT:
I have read the above comments and agree to participate in this experiment. I understand that if I have any questions or concerns regarding this project I can contact the principal investigator at the above location or the WOU Institutional Review Board at (503) 838-8271.

Name (Print) Signature Date

# Appendix E

## Debriefing Form

Thank you for your participation in the second stage of this study. The purpose of the tests completed was to assess your cognitive skills in comparison to that of other participants. You were selected from the initial pool of participants due to your rate of consuming foods containing omega-3 polyunsaturated fatty acids. You either had a high rate of consumption or a low rate of consumption. The study hypothesized that participants with a high rate of consumption would perform better on the cognitive tests.

In the conclusion of the experiment, all participants of the study will receive a debriefing email explaining in detail the purposes and results of the study.

If this study induced any degree of unnecessary stress, or if you feel any degree of discomfort after your participation in this study, please do not hesitate to contact the WOU Counseling Center at 503-838-8396.

If you have any questions about the results of the study please contact the researcher Justin Karr at jkarr06@wou.edu.

# Appendix F

The experiment in which you participated in early 2009 has now concluded all stages of investigation, and the researcher would like to thank you for your participation and offer an overall explanation of the experiments hypothesis and results.

The researcher hypothesized that the frequent consumption of omega-3 polyunsaturated fatty acids (n-3 PUFA) in the diet would better a participant's performance on a series of cognitive tests. The researcher initially administered a food frequency questionnaire to collect data regarding each participant's consumption rate of various foods containing omega-3 fatty acids, then analyzed the data and contacted the participants with the highest and lowest consumption rates. These participants proceeded to complete a series of cognitive tests to assess and compare their cognitive abilities. Previous research suggests that n-3 PUFA in the diet of pregnant women increases the cognitive and developmental abilities of their children. Also studies have shown a relationship between high n-3 PUFA consumption (through a diet of fatty fish) and decreases rates of memory problems at older age. Recently studies have also demonstrated benefits on n-3 PUFA supplementation on cognitive skills. The results of the study in which you participated found no significant relationship between n-3 PUFA consumption and performance on cognitive tasks. Though the findings of this study did not demonstrate a benefit to n-3 PUFA, certain errors could account for such findings and other researchers have previously found important benefits to n-3 PUFA in the diet.

If this study induced any degree of unnecessary stress, or if you feel any degree of discomfort after your participation in this study, please do not hesitate to contact the WOU Counseling Center at 503-838-8396.

Thank you once again for your participation in this study, and if you require any more information regarding this experiment, please contact the researcher, Justin Karr, at jkarr06@wou.edu.