THE EFFECT OF OMEGA-3 FATTY ACID SUPPLEMENTATION AND TRANSCRANIAL DIRECT CURRENT STIMULATION ON PLASMA CYTOKINES

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ABSTRACT

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THE EFFECT OF OMEGA-3 FATTY ACID SUPPLEMENTATION AND TRANSCRANIAL DIRECT CURRENT STIMULATION ON PLASMA CYTOKINES

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The purpose of this study was to examine the effect of omega-3 polyunsaturated fatty acids (n-3 PUFA) and Transcranial direct current stimulation (tDCS), on plasma inflammatory cytokines as an assessment of potential neuro inflammation. Migraine is a common disabling brain disorder influencing physical, mental, and social dimensions of quality of life. Typically, brain inflammation and migraine treatment are through pharmacologic means that often do not have beneficial outcomes. N-3 PUFA ingestion benefits brain development and function. tDCS modulates neuronal excitability by altering the resting membrane potential of a stimulated neuron, leading to a brain benefit. We hypothesized that intake of n-3 PUFA and tDCS sessions, alone or in concert, may balance the pro- and anti-inflammatory cytokines related to brain inflammation and migraine leading to a reduction in symptoms.

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CHAPTER I

INTRODUCTION

Definition of migraine

Migraines are a common disabling brain disorder characterized by severe headaches occurring most typically in the age range of 25 to 55 years old. Migraine is a worldwide disease affecting 28 million people in the US with a prevalence of 13% in males and 33% in females (Karla & Elliot, 2007, Ren, et al, 2018). The annual treatment cost for migraines in the US in 2005 totaled over $17 billion, excluding any health benefits and insurance cost (Golberg, 2005). Migraines are a chronic neurological disorder characterized by paroxysmal episodes of headache and associated symptoms typically lasting 4–72 hours (Karla & Elliot, 2007). Current migraine and pain treatment and prevention options included pain relievers, lifestyle changes, alternative medicine, and trigger avoidance. The intention of treatment and prevention options are to intent to improve the quality of life and decrease migraine symptoms. The two types of treatments for migraine symptoms and headache pain are specific and nonspecific within pharmacologic and nonpharmacologic categories. Pharmacologic therapies include antiemetics, nonsteroidal anti-inflammatory drugs (NSAIDS), analgesics, muscle relaxers, lidocaine, botox, and steroids; while nonpharmacological migraine management may involve self-relaxation, massage, or acupuncture (Karla & Elliot, 2007). The current study research is intended to identify a potential alternative treatment for trigger and symptom reduction for people who suffer from migraine.

Pathophysiology of migraine

Although the pathophysiology of migraine is unknown, current theories suggest that a migraine is largely an inherited brain disorder associated with sterile, neurogenic

inflammation and alterations in neuronal excitability (Peterlin, et al., 2015). Serum sphingolipids, especially ceramides, are initiated with episodic migraines in women who suffer from migraines (Peterlin, et al., 2015). Ceramides primarily contain saturated fatty-acyl chains and constitute the hydrophobic backbone of all complex sphingolipids. The fatty-acyl chains are linked to C2 or to the terminal carbon atom of ceramide. In general, ceramides have very low polarity and are highly hydrophobic. Enzymes involved in ceramide synthesis reside in different subcellular compartments and different metabolic pathways Castro, et, et al., 2014).

Cytokines

Ongoing studies indicate that inflammation of the central nervous system may be controlled or reduced through the release of neuroprotective factors that include some cytokines (Bayas, et al., 2002). Cytokines are signaling proteins that are released by immune cells with specific regulatory interactions between cells and can be involved in nerve injury or nerve inflammation. Inflammation occurs in response to injury or infection or as the result of an autoimmune disease (Barnes, 2009). Cytokines act by binding to a cell receptor including a change in the function or cell phenotype and may act on the cells that secrete them or on nearby cells. Cytokines assessed in this study were brain-derived neurotrophic factor (BDNF), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), and interleukin-6 (IL-6). These cytokines are involved in increasing or decreasing brain inflammation. Many cytokines have been shown to have therapeutic effects in many diseases, including impacting the nervous system (Barnes, 2009).

Cytokines can be characterized as pro-inflammatory or anti-inflammatory. Anti-inflammatory cytokines including BDNF and VEGF are produced by activated macrophages and are involved in altering inflammatory reactions. BDNF is a member of the neurotrophin family, affecting the survival and differentiation in the nervous system. It is detected in

inflammatory infiltrates in the brain of patients with encephalitis and multiple sclerosis (Bayas, et al., 2002). VEGF is a major regulator of physiological and pathological angiogenesis. VEGF causes peripheral blood monocellular cells to produce TNF-α and IL-6. VEGF also increases vascularity at the site of inflammation, causing reactions to be more severe (Angelo & Kurzrock, 2007). The proinflammatory cytokines, TNF-α, and IL-6 are included in the regulation of the proinflammatory response. IL-6 and TNF-α are involved in the process of pathological pain. Specifically, IL-6 plays a role in neural reaction to nerve injury. TNF-α has a role in both inflammatory and neuropathic hyperalgesia (Zang & An, 2009). High levels of these cytokines may correlate with either improved BNDF and VEGF or poor prognosis if TNF- α and IL-6 are increased through their impact on angiogenic factors (Angelo & Kurzrock, 2007).

Omega-3 PUFA

Low circulating levels of ceramides have been associated with increased risk for migraine (Peterlin, et al., 2015). A potential dietary treatment for migraine that would influence ceramide levels may include omega-3 fatty acid supplementation (Peterlin, et al., 2015). Omega-3 supplementation has been shown to decrease various proinflammatory compounds, including the cytokines (Calder, 2010). Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid (PUFA) found in high concentrations in brain membrane phospholipids and is important for maintaining the normal structure, function, and metabolism of brain. DHA influences gene transcription, signal transduction, and is a precursor for anti-inflammatory products (Rapoport, 2013).

tDCS

Transcranial direct current stimulation (tDCS), a non-invasive and nonpharmacologic option, has been increasingly utilized for neuromodulation for depression, stroke, and in pain treatment (Pinchuk, et al., 2013) through the delivery of a low intensity electrical

current that facilitates neuronal activity with repeated sessions (Brunoni, et al., 2011). Evaluating tDCS as an alternative intervention modality may identify the potential for tDCS use as a therapeutic component for migraine while also determining its effect, if any, on plasma ceramide levels.

Hypotheses

The H_0 is: there will be no effect on plasma cytokine levels or cytokine composition after omega-3 supplementation and/or tDCS interventions. Omega-3 supplementation alone will cause an increase in plasma cytokine levels and composition. tDCS alone will cause an increase in plasma cytokine levels with no effect on cytokine composition. Plasma cytokine levels will be increased with omega-3 supplementation and tDCS combined.

Specific Aims

- To investigate the effects of omega-3 fatty acids on plasma cytokine levels and cytokine composition in females aged 18 to 50.
- To investigate the effects of tDCS on plasma cytokine levels and cytokine composition in females aged 18 to 50.
- To investigate the effects of omega-3 supplementation combined with tDCS on plasma cytokine levels and cytokine composition in females aged 18 to 50.

The main purpose of this investigation was to determine the comparative and additive effects of omega-3 fatty acid supplementation and tDCS on cytokines related to inflammation in females aged 18 to 50.

CHAPTER II

LITERATURE REVIEW

Migraine definition

Migraines are a common worldwide disabling brain disorder influencing all physical, mental, and social dimensions of quality of life. Migraine originates from the word *hemicrania*, meaning "half of the head" (Weatherall, 2015). The clinical features of a migraine include an intense and pulsating head pain usually localized at one side of the head and usually on the left side. Migraines can last up to 72 hours (Ren, et al., 2018). Attacks can be triggered by internal or external influences or may occur for no known reason. The level of pain potentially increases through any form of movement (Weatherall, 2015). The lifetime prevalence of migraine approximates a male to female ratio of 1:3, found in about 13% in men and 33% of women (Ren, et al., 2018). Migraine occurs primarily in adult ages from 25 to 55 years and is considered the eighth most prevalent disease in the world and the fourth most prevalent disease in women according to the 2012 Global Burden of Disease study (Weatherall, 2015). According to the American Physiological Society, the cost for migraine treatment in the US in 2017 totaled $19.6 billion.

Pathophysiology of migraine

While the pathophysiology of migraine is unknown, it is a known neurological disorder. Studies have identified as few as five episodes of migraine over a lifetime, though most suffer numbers exceed this (Ferrari, et al., 2015). Migraine attacks cause the brain to lose process input and neurological symptoms (Goadsby, et al., 2017). The diverse symptoms of migraine suggest that migraine is a complex neurological disorder. Migraines affect the hypothalamus, brainstem, cortex, and limbic systems, as well as areas of the brain involved in the regulation of autonomic, affective, cognitive, and sensory symptoms (Burstein, et al., 2015). Autonomic symptoms include nausea, vomiting, nasal/sinus congestion, diarrhea, and

frequent urination. Affective symptoms include depression and irritability while cognitive symptoms are attention deficit, difficulty finding words, and reduced ability to find familiar environments (Burstein, et al., 2015).Sensory symptoms include photophobia, phonophobia, and muscle tenderness (Burstein, et al., 2015). The initial event of a migraine is often vascular constriction. The trigger mechanism is related to hypersensitivity of the limbic cortex with the genetic predisposition being multifactorial and autosomal dominant with hundreds of different mutations (Weatherall, 2015).

Migraine attacks

A migraine attack is associated with disordered brain sensory processing, an effect that can be attributed to genetics and the environment (Goadsby, et al., 2017). A genome wide association study (GWAS) in the Women's Health study confirms that genetic factors influence migraine development (Ferrari, et al., 2015). Genetic factors are responsible for 60% of diagnosed migraine with 40% of those related to nongenetic endogenous factors such as age, gender, hormonal fluctuations, and exogenous variables (head trauma, fatigue, different sleep patterns) (Ferrari, et al., 2015). The diagnosis of migraine is based on how long the headache lasts and other comorbid conditions like depression and epilepsy. Muscle tension and inflammation also have roles in headache episodes (Weatherall, 2015).

Dietary treatment

The symptoms of migraine can be prevented and reduced through many treatment options. Treatment of migraine may include dietary, pharmacologic, and non-pharmacologic modalities. Nutrition and diet play an important role in the prevention and treatment of migraine. Perhaps the most bothersome symptom of migraine may be the associated gastrointestinal effect (Lainez, et al., 2013). There are common foods that may cause migraine episodes with strong headaches and have been attributed to foods containing nitrate (Tai, et

al., 2018), a component of food commonly found in processed foods. Tyramines have also been found to be migraine triggers and are found in foods such as fermented and aged foods including alcohols (Moffett, et al., 1972). Other foods known to trigger migraine may include those containing caffeine, along with chocolate, cheese, caffeinated beverages, foods with monosodium glutamate (MSG), and fried foods. Little to no consumption of these foods may reduce migraine headaches and episodes (Tai, et al., 2018).

Pharmacological treatment

Pharmacologic treatments include antiemetics, nonsteroidal anti-inflammatory drugs (NSAIDs) and narcotics (Karla & Elliot, 2007). While many medications are used to treat migraine, none of the pharmacologic agents have been shown to have universal treatment efficiency, and side effects often limit the increased use of a given drug, leading to treatment dissatisfaction (Ren, et al., 2018). A common symptom that affects at least 60% of migraine sufferers is nausea and vomiting. These two symptoms may lead to delayed consumption of medications due to gastrointestinal (GI) upset. Antiemetics improve intestinal motility, even when nausea and vomiting is not present. NSAIDs are effective in treating migraine episodes. NSAIDs including aspirin, ibuprofen, and naproxen and can be used to relieve both pain and the accompanying symptoms. To relieve the headache pain, higher doses of NSAIDs might be needed (Lainez, et al., 2013).

Non-pharmacologic treatments

Non-pharmacologic treatments include yoga, meditation, relaxation therapies, or those related to psychophysiological management (Karla & Elliot, 2007), and may include behavioral medicine, and tDCS. Behavioral medicine is based on biological (neurochemical), psychological (behaviors, cognitions, thoughts, and emotions), and social or environmental factors that may help in the prevention, diagnosis, treatment, and rehabilitation of migraine (Grazzi & Andrasik, 2010). tDCS modifies the membrane potential of underlying cortical

neurons and has been shown to result in a significant reduction in episode frequency and pain intensity of migraine (Puledda & Shileds, 2018). Non-pharmacological treatments may work alone or in concert with pharmacological treatments. The advantage of non-pharmacologic treatment allows an individual to focus on learning how to take care of their own health while managing their lifestyle and their headache episodes.

Cytokines definition

Cytokines are a large family of small proteins released by cells in response to a stimulus. Cytokines have specific effects on the interaction and communication between cells. Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or distant cells (endocrine action) (Zhang & An, 2007). There are several sub-families of cytokines including the lymphokines (cytokines made by lymphocytes), monokines (cytokines made by monocytes), and interleukins (cytokines made by one leukocyte that act on other leukocytes) (Zhang & An, 2007). Cytokines can function individually or in combination with other cytokines and may have multiple functions including the ability to act on different cell types (Vazquez, et al., 2015).

Cytokines function

Cytokine function is controlled by B cells, T cells, and macrophages as a part of the immune system. Each of these cell types has a distinct role in the immune system with the ability to communicate with other immune cells through secreted cytokines (Vazquez, et al., 2015). T cells are lymphocytes that function in the immune system in response to pathogens, allergens, and tumors. T cells maintain immune homeostasis and are the major drivers of many inflammatory and autoimmune diseases (Kumar, et al., 2018). T cells produce multiple cytokines upon activation (Zhang & An, 2007), while B cells play a central role in the immunopathogenesis and disease pathogenesis in autoimmunity. B cells present antigens and release cytokines that act on T cells (Hoffman, et al., 2016). B cells regulate the immune

response by secreting cytokines that inhibit disease onset or progression (Hoffman, et al., 2016), with cytokine production dependent on their differentiation state and activation condition (Vazquez, et al., 2015).

Particular cytokines are involved in inflammation and nerve injury. In healthy individuals, blood levels of cytokines are normally low, but during inflammation, cytokines levels increase (Zhang & An, 2007). In inflammation, cytokines bind to a receptor, resulting in a signal to recipient cells leading to changes in function or phenotype (Galic, et al., 2012). Many cytokines are now used as therapeutic agents in immunotherapy. Cytokines act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the human immune response (Zhang & An, 2007). When nerve injury occurs, a nerve secretes cytokines and growth factors responsible for regulating nerve regeneration and might affect certain roles of cytokines (Zhang & An, 2007).

Cytokines have several roles in the central nervous system (CNS) including regulation of sleep, neuroendocrine function, and neuronal development (Galic, et al., 2012). Neuroinflammation has an important role in the pathophysiology of different acute or chronic CNS disorders. The effects of cytokines differ during the normal state and pathological state in the CNS depending on the particular cytokines (Calabrese, et al., 2014). Cytokines are categorized as either pro-inflammatory or anti-inflammatory (Barnes, et al., 2009). Accordingly, the impact of the pro-inflammatory cytokines on neurogenesis depends on their concentration. Increases in pro-inflammatory cytokines are not only due to a direct inflammatory stimulus (infection or trauma) but could be as a result of environmental stimuli such as stress. The primary cause of any abnormal cytokine levels within the brain is the production of a pro-inflammatory state and the generation of oxidative molecules that could affect neurogenesis and neural homeostasis (Calabrese, et al., 2014).

Anti-inflammatory cytokines

Anti-inflammatory cytokines are a series of immunoregulatory molecules that control the pro-inflammatory cytokine response (Opal. & DePalo, 2000). Two anti-inflammatory cytokines reviewed in this study are BDNF and VEGF, both of which are produced by activated macrophages and are involved in altering inflammatory reactions.

Brain-derived neurotrophic factor

BDNF is one of the most widely distributed neurotrophins in the mammalian brain. Neurotrophins are a family of nerve growth factors (Kowianski, et al., 2017), synthesized in the endoplasmic reticulum of cells. BDNF and its mRNA are present in nearly all brain regions including the olfactory bulb, cortex, hippocampus, basal forebrain, mesencephalon, hypothalamus, brainstem, and spinal cord (Kowianski, et al., 2017 and Bathina &Das, 2014). The earliest function of BDNF was identified in vivo in functions that promote the survival of peripheral sensory neurons during the development of the brain (Bathina &Das, 2014). BDNF supports differentiation, neurogenesis, maturation, and survival of neurons in the nervous system with a neuroprotective effect in cerebral ischemia, hypoglycemia, and neurotoxicity. Other than neuroprotection, BDNF is involved in the control of short- and long-lasting synaptic interactions through its role in the regulation of synaptic plasticity. It influences functional and structural aspects of synaptic transmission leading to increased efficiency of signal transfer (Kowianski, et al., 2017). Recent studies confirm that BDNF is involved in nerve regeneration, structural integrity, and maintenance of neural plasticity in adult brains (Dwivedi, 2010).

To be involved in its several functions, BDNF needs to bind and activate a tyrosine kinase B (TrkB) receptor. TrkB is a messenger involved in promoting the cellular effects of neurotrophins. Several studies present strong evidence that TrkB regulates neuronal differentiation including neurite development. TrkB also plays a role in neurotrophin-

dependent calcium signaling in glial cells (Gupta, et al., 2013). Many studies have identified the involvement of BDNF and TrkB in many pathological conditions where their dysregulation leads to cellular proliferative changes as well as degenerative and behavioral changes in the brain (Gupta, et al., 2013). After BDNF binds and activates TrkB, BDNF is directly involved in maturation, plasticity, and synthesis of proteins for differentiated functioning of neurons and synaptic functioning (Dwivedi, 2010). BDNF activation of tyrosine residues results in activation of different intracellular pathways leading to neural plasticity, neurogenesis, stress resistance, and survival of the cell, suggesting flexibility of Trk receptors in pro-survival functions (Dwivedi, 2010). BDNF signaling pathways activate transcription factors that are involved in regulation of the gene expression during the encoding for proteins. For BDNF to function in neurons, the neuron must be active enough to respond to the growth-promoting action of BDNF (Bathina &Das, 2014).

It has been suggested that decreased serum BDNF levels may have a role in the pathophysiology of cognitive deficits. Through its ability to augment neurogenesis and improve synaptic plasticity, BDNF may have a role in some neurological conditions such as Alzheimer's disease, dementia, and autism. For instance, BDNF levels were significantly lower in patients with schizophrenia who had lower cognitive scores than controls, suggesting that BDNF may be involved in the pathophysiology of schizophrenia, and its associated cognitive impairment, especially immediate memory (Bathina &Das, 2014). Also, BDNF is associated with pain modulation and central sensitization. Recent studies show that BNDF in migraine and headache pathophysiology is related through interaction with calcitonin gene-related peptide (Fischer, et al., 2012).

Vascular endothelial growth factor

The formation of a vascular system is a prerequisite for vertebrate embryogenesis and involves two fundamental processes, vasculogenesis and angiogenesis (Holmesm & Zachary, 2005). VEGF is a member of a family of secreted polypeptides originally described as an endothelial cell-specific mitogen (Holmesm & Zachary, 2005). It is produced by many cells including tumor cells, macrophages, and platelets. VEGF has a key role in vasculogenesis, angiogenesis, bone formation, hematopoiesis, and wound healing (Dasthagirisaheb-Shaik, et al., 2013). VEGF has emerged as the single most important regulator of blood vessel formation in health and disease. It is also it is essential for embryonic vasculogenesis and angiogenesis and is a key mediator of neovascularization in cancer and other diseases (Holmesm & Zachary, 2005).

Angiogenesis involves the formation of blood vessels in the maintenance and structures of blood vessels. It is essential for the physiological functions of tissues and the progression of diseases (Shibuya, 2011). In angiogenesis, VEGF binds to receptors found only on endothelial cells, functioning as a major angiogenic factor in normal and pathological conditions (Sherbet, 2011). Excessive angiogenesis can enhance chronic disease states such as tumor growth and metastasis, with diseases such as ulcers and ischemic heart disease resulting due to insufficient angiogenesis with hypo-driven de-regulated VEGF expression (Park, et al., 2018). Several antiangiogenic drugs target the VEGF-VEGF regulator (VEGFR) system with the VEGF-neutralizing antibody bevacizumab, a small molecule kinase inhibitor and humanized monoclonal antibody, targeting the extracellular domain of the VEGFR (Park). It has been shown that inhibition of VEGF receptor activity reduces angiogenesis (Dasthagirisaheb-Shaik, et al., 2013).

VEGF and VEGF receptors (VEGFRs) regulate vasculogenesis, the development of blood vessels from pre-existing vessels. VEGFRs are tyrosine kinase receptors (TKRs) that carry an extracellular domain involved in ligand binding, transmembrane and cytoplasmic domains, and a tyrosine kinase domain (Shibuya, 2011). VEGF exerts its effect through binding to the tyrosine kinase domain (Hofer, 2018). VEGF binds to the VEGFR on the cell surface, stimulating cellular responses resulting in receptor dimerization and activation through transphosphorylation. When cells are deficient of oxygen, namely in hypoxia, the cell produces hypoxia-inducible factor (HIF), stimulating the release of VEGF. Thus, hypoxia may be an essential regulator of VEGF expression (Park, et al., 2018). In hypoxia, the intracellular tyrosine kinase domain is interrupted by a short peptide while the kinase domain is both a regulatory and signaling element for the receptor (Hofer, 2018).

In CNS pathologies, VEGF has a neuroprotective role. VEGF induces its neurotrophic effect through enhancement of the survival of certain cells while protecting hippocampal cells from ischemic injury. Impaired VEGF release in the spinal cord can result in motor neuron degeneration (Duffy, et al., 2013). VEGF is involved in the pathophysiology of migraine with the VEGF gene and haplotypes associated with migraines (Goncalves, et al., 2010).

Inflammatory cytokines like VEGF can enhance inflammation by upregulating important mediators of angiogenesis. The vascular changes caused by the angiogenic function of VEGF cause chronic inflammation. VEGF increases the vascularity at the site of inflammation often leading to a more severe reaction. It is clear that angiogenesis and inflammation are linked together. Blocking VEGF results in a decrease in angiogenesis' nutrient supply to migration and cytokine production by activated endothelial cells (Angelo & Kurzrock, 2007).

Pro-inflammatory cytokines

Pro-inflammatory cytokines, produced predominantly by activated macrophages, are positive mediators of inflammation and are involved in the upregulation of inflammatory reactions (Zhang & An, 2007). They play a central role in inflammatory diseases of infectious or non-infectious origin. In a wide variety of infections, cytokines are released as part of a host response due to inflammasome activation, a process known as the pro-inflammatory cytokine response. Cytokines with a proinflammatory function are elevated in infected tissue, and with time, some of them increase systemically (Makowski, 2017). Anti-inflammatory cytokines often function to control the pro-inflammatory cytokine response. Two pro-inflammatory cytokines reviewed in this study are Tumor Necrosis Factor-alpha (TNF-α) and Interleukin 6 (IL-6).

Tumor necrosis factor-alpha

TNF- α is a pro-inflammatory cytokine produced by numerous cells in the body including macrophages during acute inflammation. It is responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis (Idriss & Naismith, 2000). TNF-α, originally identified as a factor that leads to rapid necrosis of transplantable tumors, is now classified as a pro-inflammatory cytokine involved in the innate immune response. In the CNS, TNF-α exerts both homeostatic and pathophysiologic roles. In the healthy CNS, TNF-α has regulatory functions that include a role in synaptic plasticity, learning and memory, sleep, and food and water intake (Olmos & Llado, 2014). De novo production of TNF- α is an important component of the neuroinflammatory response associated with several neurological disorders. Neuroinflammation and excitotoxicity are key as triggers and sustainers of the neurodegenerative process and thus, elevated levels of TNF-α has been found in traumatic brain injury, ischemia, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis (Olmos & Llado, 2014).

TNF-α has been implicated in a diverse range of inflammatory, infectious, and malignant conditions. The importance of TNF-α in inflammation has been highlighted by the efficacy of anti-TNF-α antibodies or the administration of soluble TNF receptors (TNFRs) to control disease activity in rheumatoid arthritis and other inflammatory conditions (Bardley, 2008). TNF-α also has a role in migraines. Elevated levels of TNF-α in serum and urine have been found in migraine suffers. TNF-α stimulates transcription of calcitonin gene-related peptide, a protein with a role in migraine pathophysiology (Schurks, 2011).

TNF-α is usually undetectable in healthy individuals, but elevated serum and tissue levels are found in inflammatory and infectious conditions with serum levels correlating with infection severity. Although cells of the monocyte/macrophage lineage are the primary source of TNF-α in inflammatory diseases, a wide range of cells can produce TNF-α, including T and B lymphocytes, and endothelial cells (Bardley, 2008).

The pro-inflammatory effects of TNF-α are induced through its ability to activate NF-κB. The NF-κB pathway is a pro-inflammatory signaling pathway, largely based on its role in the expression of pro-inflammatory genes that encode for cytokines and chemokines (Lawrence, 2009). Almost all cell types, when exposed to TNF-α, activate NF-kB, leading to the expression of inflammatory genes (Sethi, et al., 2008). Over 400 genes have been identified that are regulated by NF-kB activation including cyclooxygenase-2 (COX-2), lipoxygenase-2 (LOX-2), cell-adhesion molecules, antiapoptotic proteins, inflammatory cytokines, chemokines, and inducible nitric oxide synthase (iNOS). TNF-α produced by tumor cells or inflammatory cells in the tumor microenvironment can promote tumor cell survival through the induction of genes encoding for NFkB-dependent anti-apoptotic molecules (Sethi, et al., 2008). TNF-α is a critical component for effective immune

surveillance and is required for proper proliferation and function of NK cells, T cells, B cells, and macrophage cells (Sethi, et al., 2008).

Interleukin-6

Interleukins are a member of the cytokine family that can be produced by many cells with the essential role of activation and differentiation of immune cells. As with the other cytokines, interleukins have both pro- and anti-inflammatory properties. The primary function of interleukins is in the modulation of growth, differentiation, and activation during inflammatory and immune responses (Vaillant & Ourie, 2020).

Interleukin-6 (IL-6), a cytokine originally identified as a B-cell differentiation factor almost 30 years ago, is necessary for the induction of B cell maturation into antibody-producing cells (Erta, et al., 2012). IL-6 involvement in the immune response and controls immune cell involvement in the control of other cells including T cells (Erta, et al., 2012). IL-6 is a cytokine not only involved in inflammation and infection responses, but also in the regulation of metabolic, degenerative, and neural processes. During signaling, IL-6 stimulates target cells via the IL-6 receptor. This receptor participates in many pathways including the mitogen-activated protein (MAP) and mitogen-activated protein kinase (MAPK) pathway (Scheler, et al., 2011). IL-6 is produced at the site of inflammation with a key role in the acute phase response as defined by a variety of clinical and biological features such as the production of acute-phase proteins (Gabay, 2006).

IL-6 in vivo has different actions, including being an activator of the immune system. IL-6 plays a role during the transition from innate to acquired immunity. Acute inflammation is defined as filtering of neutrophils, which is then replaced by monocytes and T cells in order to prevent increased tissue damage from inflammation (Scheler, et al., 2011). A study examined the effect of IL-6 on chronic inflammation in mice using an IL-6 gene knockout

mouse identified an anti-inflammatory component attributable to the action of IL-6 in both local and systemic acute inflammatory responses. IL-6's function during the acute response is to suppress pro-inflammatory cytokine release without compromising the level of anti-inflammatory cytokines. Therefore, IL-6 may act as an anti-inflammatory cytokine where it can have a protective effect during acute inflammation where during chronic inflammation, it acts as a pro-inflammatory cytokine (Gabay, 2006).

IL-6 action leads to a reduction in the production of fibronectin, albumin, and transferrin, while reducing gene expression (Tanaka, et al., 2014 and Dendorfer, et al., 1994). IL-6 gene expression is regulated by prostaglandins and their second messenger cyclic AMP following immune cell stimulation with lipopolysaccharide (Dendorfer, et al., 1994). IL-6 also plays a synergistic role in regulating IL-6 gene expression. Single-nucleotide polymorphism (SNP) is a promoter of the IL-6 gene resulting in transcription and expression (Lu & Zheng, 2016). Dysregulation of IL-6 gene expression has been associated with the pathogenesis of immunological related diseases (Faggioli, et al., 1997).

IL-6 is involved in the process of signaling tissue damage, indicating the occurrence of an emergency. This emergency role is a part of the strict regulation of IL-6 synthesis at both gene transcription and post-transcription (Tanaka, et al., 2014). IL-6 levels increase in the plasma of patients suffering from a traumatic brain injury and inflammation (Erta, et al., 2012). In the initial stages of inflammation, IL-6 is synthesized and released to the bloodstream where it moves to the liver resulting in a rapid induction and release of an extensive range of acute-phase proteins including C-reactive protein (CRP), serum amyloid A (SAA), and fibrinogen (Tanaka, et al., 2014).

Polyunsaturated Fatty Acids

Definition of PUFA

Polyunsaturated fatty acids (PUFAs) are an important component of phospholipids which are fundamental components of cells, tissues, and organs while also being used in the synthesis of biologically active substances (Sokala, et al., 2018). PUFAs are essential fatty acids that cannot be produced by the human body, and therefore, need to be consumed in the diet. PUFAs have varying beneficial essential and critical roles in the human body. They play a key role in cardiovascular and inflammatory diseases and ensure an optimal environment for membrane protein function, maintaining membrane fluidity, and influencing lipid raft formation in the cell membrane (Calder, 2010). Changes in membrane phospholipid fatty acid composition can influence the function of cells involved in inflammation with alterations in membrane composition influencing raft structure and possibly changing gene expression (Calder, 2010).

Structure of PUFA

The structure of a PUFA is characterized by the presence of carboxylic acid (COOH) at one end of the molecule, a methyl end (-CH3) at the other end, and the requirement is to have at least two double bonds in the chain (Bentsen, 2017). Polyunsaturated fatty acids are identified based on the number of carbons present in their chains and the number of double bonds. Particularly the PUFAs related to this study include linoleic acid (LA; C18:4); arachidonic acid (AA; C20:4); eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6; see Figure 1). Omega-3 fatty acids (n-3) have their initial double bond on the third carbon atom from the methyl or omega end of the fatty acid while omega-6 fatty acids (n-6) have their first double bond on the sixth carbon atom from the methyl terminus (Bentsen, 2017).

Desaturase and elongase

Humans require the dietary essential fatty acids linoleic acid (LA) and alphalinolenic acid (ALA) as the building block to synthesize other PUFAs. Each PUFA can be consumed in the diet or can be synthesized from precursors through the participation of different desaturases in conjunction with elongases (Bentsen, 2017). A Desaturase is an enzyme that abstracts two hydrogens from a fatty acid to introduce a carbon/carbon double bond toward the carboxyl end of the molecule. Elongase is an enzyme that catalyzes the carbon-chain extension of a fatty acid through the addition of two carbons to the molecule (Yilmaz, et al., 2017). LA is a precursor n-6 fatty acid for longer fatty acids while ALA is the precursor for longer-chain n-3 fatty acids. The more LA present in the diet, the less ALA converted into longer-chain n-3 PUFA. On the other hand, decreased dietary intake of LA has a potential to increase EPA and not AA in tissues as a result of de novo synthesis from ALA. While LA is converted into AA, this conversion is low. AA in the diet is dependent on meat and poultry intake levels. The numerous double bonds in AA provide mobility, flexibility, and fluidity to membranes. By controlling membrane fluidity, AA influences the function of specific membrane proteins involved in cell signaling. Other roles of AA include neuron function, brain synaptic plasticity, and long-term potentiation in the hippocampus

(Tallima & El, 2017). Increasing dietary n-3 intake has been shown to decrease AA levels in the human body (Bentsen, 2017).

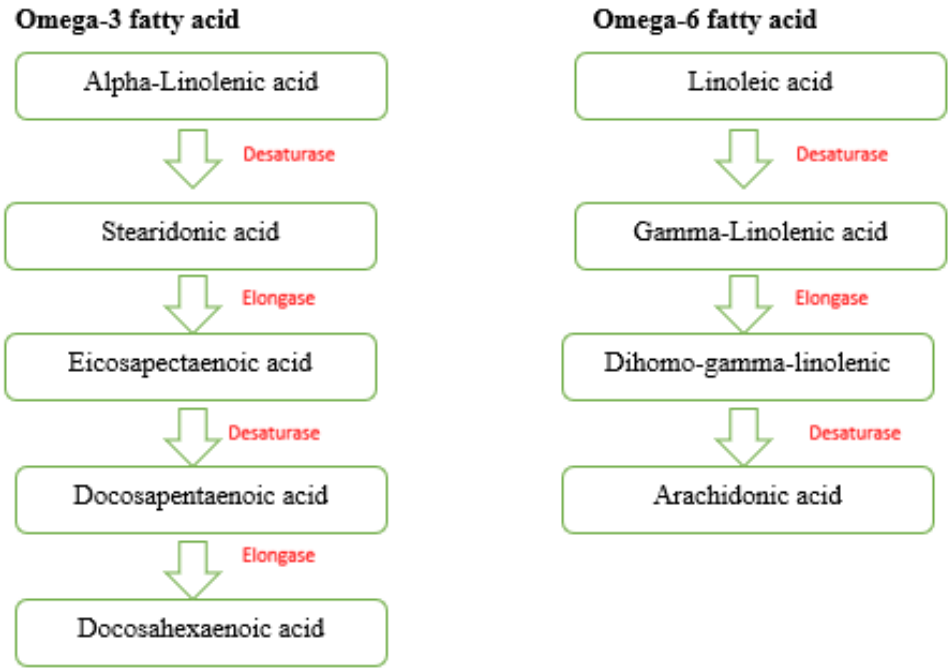


Figure 1. Polyunsaturated fatty acid metabolism

Omega-3 PUFA

Omega-3 (n-3) polyunsaturated fatty acids (PUFA) are a family of polyunsaturated fatty acids that are characterized by their double bond structure (Calder, 2010). N-3 PUFAs are required for general human health, especially for brain development and function (Blondeau, et al., 2015). N-3 PUFAs reduce the development of symptoms in many diseases such as diabetes, Alzheimer's disease, inflammatory diseases, and migraine headaches (Zivkovic, et al., 2011). High n-3 in plasma are associated with reduced risk of neurodegenerative diseases (Zivkovic, et al., 2011).

Linoleic acid and alpha-linolenic acid

The most abundant PUFAs found in the human brain are AA and DHA. They make up the one-fifth of dry brain weight. EPA, while present in the brain, is found at a lesser amount due to its extensive metabolism. AA and DHA are required for the development and function of the brain. The amount of EPA found in the brain is 250 to 300 times lower than DHA (Bentsen, 2017). Consumption of EPA and DHA has many beneficial effects in the human body, where they are the precursors of several metabolites that are potent lipid mediators

involved in the prevention or treatment of several diseases (Swanson, et al., 2012). Dietary sources of the PUFA differ depending on the PUFA. LA is found in seeds, sunflower oil, soya, and wheat. AA is found in animal sources like eggs and meat. ALA is found in flax, chia, and nuts. The most abundant source of EPA and DHA is fish and fish oil (Bentsen, 2017). The amount of EPA and DHA in a food may be influenced by food processing and different food sources. Industrial food production tends to increase the n-6/n-3 ratio (Bentsen, 2017). The original ratio of the human diet was 1:1 to 2:1. In a typical Western diet, the ratio is currently 10 to 20:1. This current imbalance indicates that the amount of dietary n-6 PUFA should be reduced and the n-3 PUFA amount should be increased (Bentsen, 2017). According to current dietary guidelines, the daily recommended intake of EPA+ DHA is at least 250mg with a total intake of n-3 PUFA at 1.1 to 1.6g/day. The levels of EPA and DHA present in the brain are often a reflection of the specific phospholipids present and lipids involved in diet composition. Less EPA in the brain reflects intake and EPA elongation to DHA. DHA is enriched in phosphatidylethanolamine (PE) and phosphatidylserine (PS) whereas EPA is preferably esterified to phosphatidylinositol (PI) (Dyall, 2015). The level of EPA and DHA in the brain is lower than plasma but is reflective of plasma unesterified fatty acid pool. The brain maintains EPA and DHA levels via uptake from the plasma reflective of dietary and/or liver sources (Dyall, 2015).

DHA

DHA is found in most tissues including the myocardium, retina, and brain. It is required for the development of the sensory, perceptual, cognitive, and motor neural systems during brain growth (Kidd, 2007). The neurons are continually forming axons with their accompanying cell membranes. The growing membrane is relatively fluid attributable to DHA and its high degree of unsaturation. Even the synapses, the primary functional units of brain circuits, consist of membranes that are enriched with DHA (Kidd, 2007). DHA has a greater

potential to affect cell signaling than EPA due to its tendency to accumulate in sphingomyelin/cholesterol-rich rafts at levels higher than that seen with EPA (Dyall, 2015).

EPA

EPA and DHA are essential for proper fetal development and healthy aging [62]. EPA is found in cholesterol esters, triglycerides (TG), and phospholipids while DHA is found in phospholipids. EPA is important for the brain's development (Ellulu, et al., 2015) with colostrum and breast milk containing more EPA than DHA (Kidd, 2007). EPA is considered to be an anti-inflammatory PUFA whereas, DHA is considered to be a gene regulator and anti-inflammatory PUFA (Calder, 2010). DHA and EPA can provide a degree of biochemical backup for each other. Although EPA may not be readily desaturated and elongated to DHA, DHA can be easily retro converted to EPA (Kidd, 2007). The human body converts close to 5% of ALA to EPA (Ellulu, et al., 2015).

Proectins, and eicosinoids

EPA and DHA are important substrates for the synthesizes of lipid mediators like the protectins through the action of the cyclooxygenase enzymes and are important for the resolution of the inflammatory process (Calder, 2010). The immunomodulatory effects of n-3 PUFA are related to the amount and types of eicosanoids made from AA and EPA (Yui, et al., 2015). Eicosanoids are lipid mediators [66] that perform numerous regulatory functions in the brain and the rest of the body, especially in the regulation of immune and inflammatory responses (Yui, et al., 2015). Eicosanoids regulate a wide variety of physiological responses and pathological processes. They control important cellular processes, cell proliferation, apoptosis, metabolism, and migration. Eicosanoid biosynthesis is usually initiated by the activation of phospholipase A2 (PLA2), which then acts on membrane phospholipids (Yui, et al., 2015). PLA2 plays a central role in lipid signaling and a variety of inflammatory conditions. PLA2 cleaves the sn-2 ester bond of phospholipids, releasing a free FA. The

produced free FAs are primarily AA (Balsinde & Dennis, 2010). The number of FAs released determines the type of eicosanoid produced (Ricciotti & FitzGerald, 2011).

Prostaglandins

Prostaglandins (PGs) are eicosanoids that can be produced by nearly all cells in the body (Bagga, et al., 2003). PGs are bioactive lipids derived from particular PUFAs and have important roles in metabolism and immunity (Park, et al., 2006). PGs are not stored in cells but are synthesized constitutively in response to trauma, stimuli, or signaling molecules (Park, et al., 2006). They play a key role in generating an inflammatory response (Bagga, et al., 2003). PGs are synthesized when AA is released from the plasma membrane by phospholipases and is metabolized by cyclooxygenase (COX) (Bagga, et al., 2003 and Park, et al., 2006). Following biosynthesis, PGs will increase in inflamed tissue and promote the development of the cardinal events of acute inflammation including swelling, pain, and redness (Bagga, et al., 2003) which may or may not be a beneficial response. To decrease the negative symptoms caused by PG synthesis, intake of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) has been increased. NSAIDs inhibit the production of PG by blocking the COX enzymes, in turn suppressing inflammatory pain in an individual (Hawkey, 2001). While NSAIDs decrease pain, they may also lead to damage in the human body in locations including the gastrointestinal tract, resulting in mucosal damage, ulceration, and ulcer complications in the GI tract (Hawkey, 2001).

Prostaglandins' role

PGs have important roles in the human body including the maintenance of cell development and homeostasis (Yang, et al., 2014). The most abundant prostaglandin in the human body is Prostaglandin E2 (PGE2) with its homeostatic, inflammatory, or anti-inflammatory effects. Inhibition of PGE2 synthesis has been the target of many anti-inflammatory strategies (Park, et al., 2006). PGE2 has a role in pain sensation, and

inflammation. PGE2 acts on neurons and contributes to the systemic response to inflammation including a role in fever, fatigue, and pain hypersensitivity (Zarghi & Arfaei, 2011). The precursor of PGE2 is AA. To decrease the synthesis of PGE2, EPA+DHA can be inserted into a phospholipid resulting in displacement of AA from a tissue phospholipid with the potential for increased PGE3 synthesis (Bagga, et al., 2003). In ex vivo studies, PGE3 has been found to have anti-proliferative and anti-inflammatory properties while reducing the proinflammatory effects of PGE2 (Yang, et al., 2014). EPA serves as the substrate for PGE3 synthesis via the action of the COX-2 enzyme (Bagga, et al., 2003).

Cyclooxygenase 1 and 2

The cyclooxygenase enzymes are present in many cell types (Tan, et al., 2018). It is a bifunctional enzyme that contains both cyclooxygenase and peroxidase activity with a minimum of two distinct isoforms known as COX-1 and COX-2 (Ricciotti & Dennis, 2010) Recent studies have shown the relationship between COX-1 and COX-2 is not straightforward, where COX-1 and COX-2 are genetically independent proteins. The genes in the human body for COX-1 and COX-2 are located on different chromosomes and show different properties (Zarghi & Arfaei, 2011). COX-1 is constitutively expressed commonly in most cells and it is a dominant source of prostanoids that serves in homeostasis, gastrointestinal protection, and general housekeeping (Bagga, et al., 2003 and (Hawkey, 2001). COX-2 is an inducible enzyme with its production induced by an inflammatory stimulus, hormones, and growth factors. The most critical function of COX-2 is as a source of prostanoid formation in inflammation and proliferative diseases (Bagga, et al., 2003). It is not detected in most normal tissues, but its expression is a result of stimuli from compounds such as pro-inflammatory cytokines like TNF-α, lipopolysaccharides, growth factors, hormones, or may be a result of disorders of water-electrolyte hemostasis, with the result being an increase in synthesis of prostaglandins in inflamed and neoplastic tissues (Zarghi & Arfaei, 2011).

EPA, DHA, and NFkB

N-3 PUFAs are beneficial in autoimmune disorders, inflammatory diseases, and heart disease. EPA and DHA reduce the development of central nervous system diseases by reducing or balancing the concentrations of cytokines (Yamagata, 2017). Diets low in n-3 intake and high in n-6 intake are believed to be problematic in pro-inflammatory conditions as AA is metabolized to eicosanoids that induce pro-inflammatory cytokine production. Increased intake of EPA and DHA has been found to reduce the amount of AA available for eicosanoid synthesis. Following ingestion, EPA and DHA enter the bloodstream and are incorporated into cell membrane phospholipids (Yamagata, 2017). The release of EPA and DHA from tissues inhibits the activation of the nuclear factor κ B (NFκB) responsible for increases in the expression of pro-inflammatory cytokine genes by cells involved in inflammatory processes. The resulting inflammatory cytokines are small signaling proteins synthesized by a range of cells that are activated during inflammatory responses, in turn regulating inflammation through a complex network of interactions (Yamagata, 2017). In aging, an increase in levels of circulating pro-inflammatory cytokines versus anti-inflammatory cytokines promotes chronic systemic inflammation. Circulating pro-inflammatory cytokines can travel to the brain with the assistance of specific transporters, creating problems attributable to an increased vulnerability of the blood-brain barrier to prolonged brain inflammation (Yamagata, 2017). As mentioned earlier, this study focused on the cytokines BDNF, VEGF, TNFα, and IL-6.

BNDF and n-3 PUFA

BDNF affects energy metabolism following intracerebroventricular administration, leading to a decrease in energy intake and body weight loss. This action of BDNF is associated with nerve cell survival and adaptive plasticity (Bathina & Das, 2014). BDNF has a positive correlation with low-density lipid (LDL) cholesterol, total cholesterol, and

triglycerides. Consuming n-3 PUFA supports those functions of BDNF. BDNF treatment of diabetic animals resulted in decreased plasma glucose, non-esterified fat, phospholipids, and liver weight (Bathina & Das, 2014).

Dietary n-3 PUFAs normalize BDNF levels, reduce oxidative damage, and improve learning ability after traumatic brain injury. These results emphasize the possible interaction between dietary n-3 PUFA and BDNF that may be relevant to brain recovery [31]. BDNF facilitates synaptic transmission and regulates gene expression by increasing levels of synapsin I and CREB release, helping in the maintenance of synaptic plasticity. A study with knockout mice with reduced BDNF levels were found to develop hyperphagia, obesity, and resistance to insulin and leptin (Bathina & Das, 2014).

VEGF and n-3 PUFA

VEGF plays a key role in vasculogenesis and angiogenesis and stimulates vascular permeability, leading to inflammation (Dasthagirisaheb-Shaik, et al., 2013). DHA inhibits VEGF-induced endothelial cell migration, which plays a role in angiogenesis and wound repair. DHA reduces VEGF-induced cell migration, which is mediated by a specific signaling pathway called FFAR4 and the PP2A/ERK1/2/eNOS (Balogun et al., 2013).

IL-6, TNF-α and n-3 PUFA

IL-6 works as a part of the acute phase response as defined by a variety of clinical and biological features such as the production of acute-phase proteins (Gabay, 2006). TNF-α has both homeostatic and pathophysiological roles. In the healthy CNS, TNFα has regulatory functions. During pathological conditions, TNF-α regulates apoptosis. N-3 PUFAs significantly reduce markers of inflammation through inhibition of TNF-α induced activity of NF-κB (Flock, et al., 2014). Higher amounts of DHA levels lead to a reduction of TNF-α concentrations while higher AA levels are associated with higher TNF-α concentrations. Flock et al. found acute elevations in TNF-α may improve the sensitivity to specific

inflammatory conditions (Flock, et al., 2014). EPA and DHA can inhibit the production of inflammatory proteins such as TNF-α and IL-6 in various cell types including endothelial cells, monocytes, macrophages, and dendritic cells (Naegel, et al., 2018).

Several studies have shown that n-3 PUFA in vitro inhibits the production of pro-inflammatory cytokines from macrophages and has been shown to have a beneficial inhibitory effect in autoimmune diseases in vivo (Naegel, et al., 2018). DHA, through targeting the lipopolysaccharides (LPS) surface receptor, suppresses NFkB activity and the production of inflammatory cytokines in microglia (Naegel, et al., 2018). Some of the anti-inflammatory effects of DHA are purported to be mediated through its metabolite NeuroprotectinD1, shown to inhibit the expression of cytokines induced by Aβ peptides in microglia (Naegel, et al., 2018). N-3 PUFAs are used to synthesize resolvins, a factor that inhibits the production of inflammatory cytokines in microglial cells and has anti-inflammatory effects. A study with rats found that resolvins inhibit the expression of inflammatory cytokines like TNF-α and IL-6, resulting in a reduction of inflammation and pain (Naegel, et al., 2018).

Transcranial Direct Current Stimulation

tDCS definition and function

tDCS has been known to induce long lasting alterations in cortical excitability both in experimental animals and humans. tDCS is a non-invasive, and safe, and painless technique that has been applied to various pain disorders and may be a cost-effective and long-term treatment option for migraine. It modulates the activity of in the brain regions (Rueger, et al., 2012) and establishes brain behavior relationships across a variety of cognitive, motor, social, and affective domains. It has been used as a reversible neuromodulation technique to study the behavioral impact of distinct neural networks in experimental animals. This noninvasive brain stimulation appears to enhance motor learning in healthy volunteers. Recent studies have tried

to identify its therapeutic potential for various neurological and psychiatric disorders. Studies in human stroke patients have shown tDCS to be a promising therapeutic intervention to ameliorate motor deficits, aphasia, and neglect, suggesting that tDCS may become a new adjuvant tool to promote recovery of function after stroke (Thair, et al., 2017).

Although tDCS is already widely used in human studies, the basic mechanisms of its action remain incompletely explored unclear, and details about the neurobiological effects of tDCS are still unknown. The long-lasting effect on cortical excitability outlasting the actual stimulation seem to depend on alterations of resting membrane potentials and synaptic plasticity (Thair, et al., 2017).

Migraine and tDCS

Migraine is a chronic disorder that causes major headaches. There are several treatment options for migraine including the use of NSAIDS along with other pharmacological and non-pharmacological means. There has been recent interest in therapeutic modalities using transcranial weak electrical stimulation through scalp electrodes via tDCS to understand its ability to modify brain function as a possible treatment for psychiatric conditions. A range of electrotherapy paradigms have been investigated, but no consistent method has reported reproducible stimulation “dosage” (Bikson et al., 2016). The usage of tDCS for migraines may be different from other brain diseases and disorders. To date, no studies have examined combined tDCS and n-3 PUFA on migraine or brain inflammation. This research will fill this gap by identifying a n-3 PUFA and tDCS combination therapy in an attempt to elucidate an effective long-term, cost-effective, and beneficial treatment.

CHAPTER III

METHODS

Study Design

Population of the study

Female adults with or without migraine between the ages of 18 to 50 years of age were recruited from Texas Woman's University (TWU) staff, students, and through online recruitment (see Figure 2). Inclusion criteria were, female, not utilizing omega-3 fatty acid supplements in the last 3 months, not pregnant, and able to visit the Pioneer Performance Clinic at TWU for blood isolation. Possible subjects were excluded if they were diagnosed with any chronic disease or taking migraine medications. Other details of the study recruitment and screening have been reported previously within the consent form. The study was approved by the TWU Institutional Review Board.

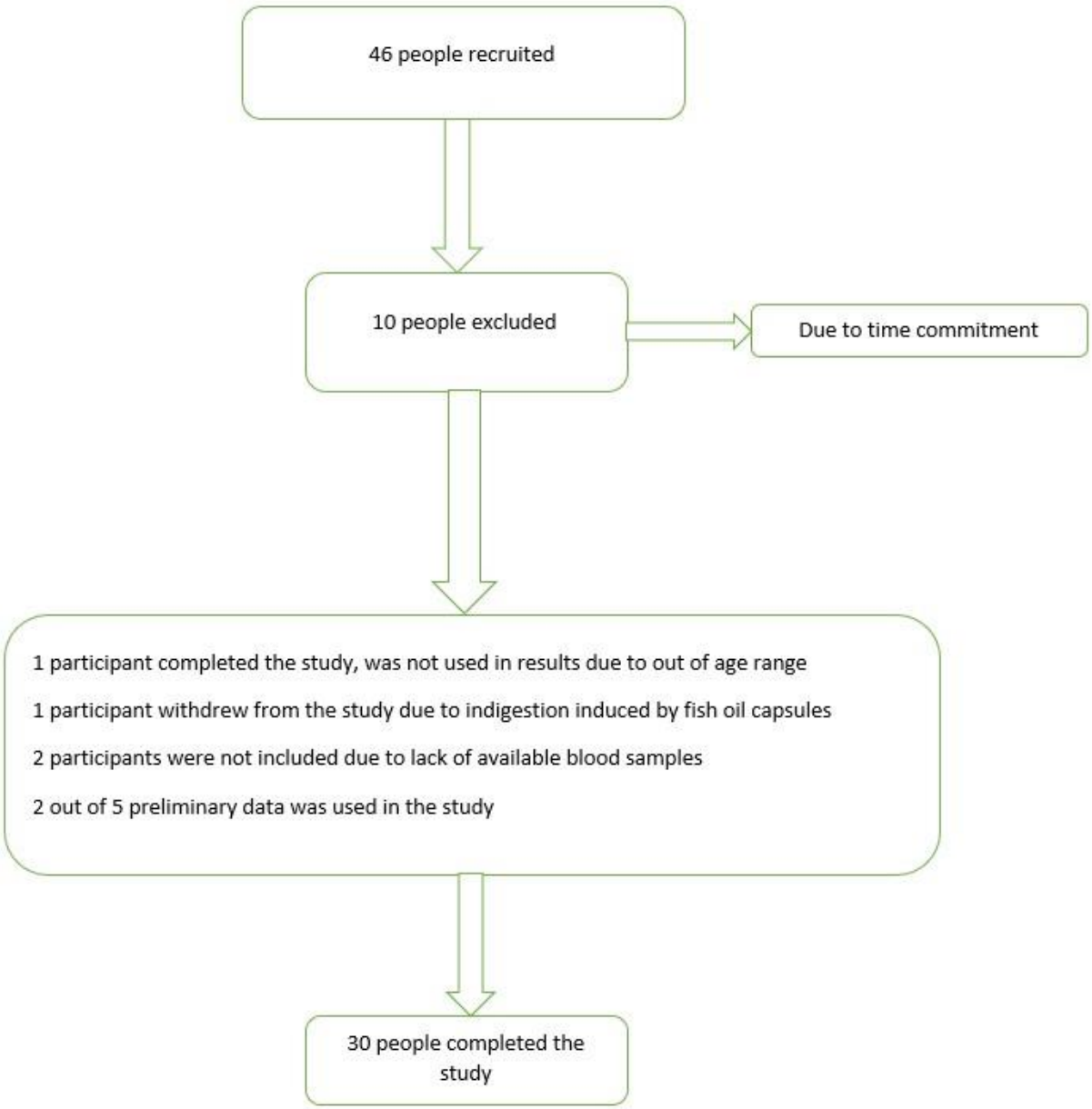


Figure 2. Participant recruitment

Treatments in this study

The treatments for this study were n-3 PUFA, tDCS or combines n-3PUFA/tDCS. Ingestion of fish oil as two soft gels of fish oil daily provided 2,400mg of total n-3 fatty acids at 636 to 720mg EPA and 408 to 480mg DHA per capsule. Recommended intake of n-3 PUFA is 1.1g for female adults and 1.6g for male adults as ALA.

Each participant was randomly assigned to one of the three treatment groups. Group one was placed on the n-3 PUFA supplement for 6 weeks. Following informed consent,

participants were informed to consume two soft gels at the same time daily until study completion. Participants were provided with all n-3 PUFA supplements at study onset. Whole blood was collected three times over the duration of the study, at baseline, midpoint (last day of the fourth week), and the final day (the last day of the sixth week).

tCDS only group received tDCS treatment five times weekly for 4 weeks. Each treatment lasted for 30 minutes. The two electrodes of tDCS are located on the left and right sides of the scalp. To use tDCS as a treatment, it requires no skin damage and excessive hair product. Placing the electrode ends into a sponge and using a saline solution to wet the sponges is required. After the electrodes are located on the scalp, the duration, and current intensity are programmed based on individual tolerance [86]. While receiving tDCS treatment, participants were able to eat, talk, study, and read. Baseline blood samples were obtained on the first day of treatment with the final blood draw occurring following the final tDCS treatment. tDCS treatment level was based on participants' tolerance with participant permission to increase the tDCS dosage after their second session.

The n-3 PUFA and tDCS combination lasted for a 6-week period. Participants were informed about the schedule as a part of the informed consent form. Participants consumed encapsulated n-3 PUFA supplements for the entire 6week period. For the final 2 weeks of the 6-week period, tDCS treatment was added to the treatment protocol. The n-3 PUFA supplement and protocol was consistent with that of the n-3 PUFA only treatment. For the final 2 weeks of the study, participants came to the Pioneer Performance Clinic for the additional tDCS treatment. As with the tDCS treatment group, each tDCS session was 30 minutes long with the same limitations as the tDCS only group. Blood was isolated three times, baseline right after participant signature of the informed consent at which time the n-3 PUFA supplement was provided. The second blood draw was on the last day of the fourth

week prior to tDCS initiation. The final blood draw occurred at the termination of the study at the end of week 6.

Blood sample collection

A total of 30 participants completed the study. At the time of blood collection, two 5ml tubes with a heparin anti-coagulant were drawn. Tubes were centrifuged at 3000xGRP for 15 minutes to allow for isolation of plasma. Erythrocytes were frozen for eventual preparation of erythrocyte ghosts for phospholipid analysis, and plasma was aliquoted into three fractions and frozen at -80ºC for subsequent analysis. The inflammatory biomarkers BDNF, VEGF, IL-6, and TNF- α were evaluated in plasma using sandwich ELISA kits (MilliporeSigma Corporation Billerica, MA, USA 01821). All samples and standards were measured in duplicate according to the kit protocols. Following standards and plasma sample addition to ELISA plate wells, the assay is incubated. Following all incubation and antibody additions, Horse Radish Peroxidase-Streptavidin is added for color development. Following the addition of stop solution, the absorbance was read at 450nm. For plasma samples with high analysis concentrations, it was necessary to dilute the wells for analysis with diluent B that was provided in the ELISA kits.

Statistical analysis

After running the ELISA plates for each cytokine, analysis was done with the help from the Center for TWU Research Development and analyzed using SPSS repeated measured ANOVA with p-value < 0.05.

CHAPTER IV

RESULTS

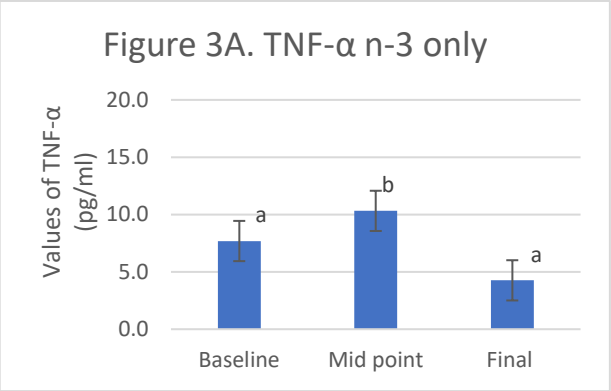
Participant Characteristics

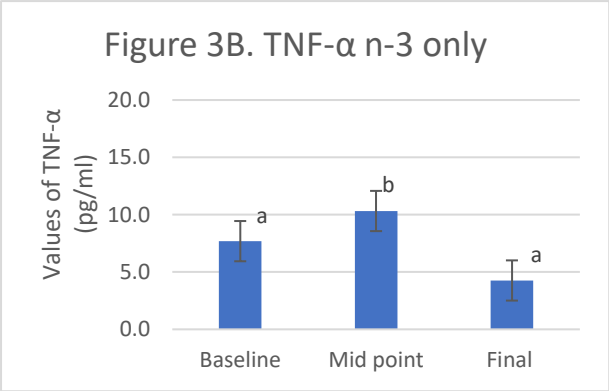
This study was conducted in the Pioneer Performance Clinic on TWU Denton campus. A total of 35 female participants were recruited. One person withdrew from the study due to gastrointestinal discomfort from the intake of the n-3 soft gels, and four individuals withdrew prior to starting because of scheduling. Thirty-one women participated in and finished the study. One participant was excluded as she exceeded the age limit. This study had three treatment groups, with variations in participant numbers. The n-3 only group had 11 participants, the tDCS only group had 10 participants, and the n-3 and tDCS combination group had nine participants. Participant demographics are provided in Table 1 below.

Table 1

Participants information (±SD)

Groups	Omega-3 only	tDCS only	Combination
Age	28±6.5	33.3±8.9	28.9±8.2
Weight	143.4±16.4	126.1±15	121.1±15.3
Height	64.8±1.3	65.2±1.3	65.6±1.1
BMI	24.1±3.4	20.8±2.4	20.8±4.1





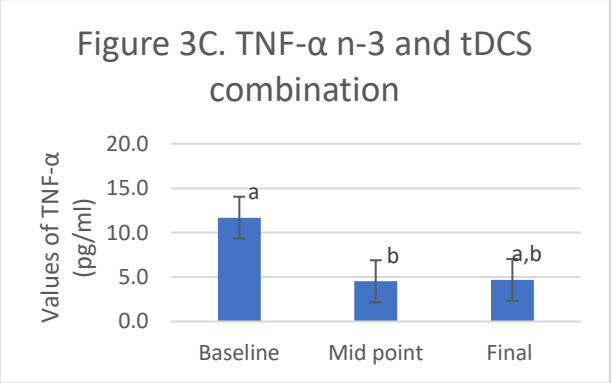


Figure 3 (A, B, and C). ±SEM of TNF-α with three different treatment groups. Note. Figure 3A shows TNF-α levels are baseline, midpoint, and final blood draws with n-3 treatment. Figure 3B shows TNF-α levels in baseline and final blood draws with tDCS treatment. Figure 3C shows TNF-α levels in baseline, midpoint, and final blood draws with combination of n-3 and tDCS treatments. (a and b in the figures stand for statistical difference).

Following n-3 PUFA, tDCS only, and n-3 PUFA and tDCS combination treatment, TNF-α levels were evaluated. Figures 3A, 3B, and 3C are reported as average ±SEM TNF-α levels separated by treatment group. In Figure 3A, there was a 34% increase in TNF-α between baseline and the midpoint with n-3 PUFA ingestion only. Between the 4-week midpoint and the final 6-week timepoint, there was a 59% decrease in TNF-α levels. Between the baseline and the final timepoint there was a pattern of decreased TNF-α levels with n-3 PUFA ingestion. Though it appears that there was a 45% reduction, the change was not significant. In tDCS only treatment, there was a 56% decrease between baseline and the final timepoint (see Figure 3B) at 4 weeks. In the combination treatment, there was a 61% decrease between the baseline and midpoint with only n-3 PUFA ingestion, that remained unaltered with the addition of tDCS over the final 2 weeks of the 6-week treatment period. Surprisingly,

in the N-3 PUFA only and n-3 PUFA and tDCS combination group when receiving the same n-3 PUFA treatment, they had an opposite response at the midpoint blood draw. The baseline of the n-3 PUFA and tDCS combination group's average was 52% higher than the n-3 PUFA only group's baseline average and was equivalent the n-3 PUFA only midpoint. The midpoint in n-3 PUFA and tDCS combination group's midpoint average TNF-α was 56% lower than the n-3 PUFA only treatment group midpoint. Interestingly, by the end of the 6-weeks of n-3 PUFA ingestion, both the n-3 PUFA only and combination group achieved the same TNF-α level, that being approximately 40% of their highest average levels.

Table 2.

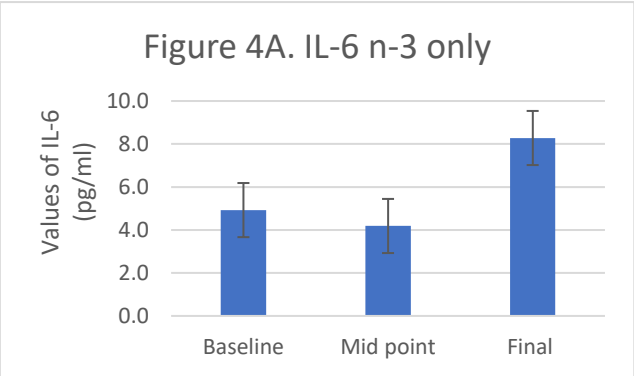
Statistical analysis of TNF-α levels in three groups

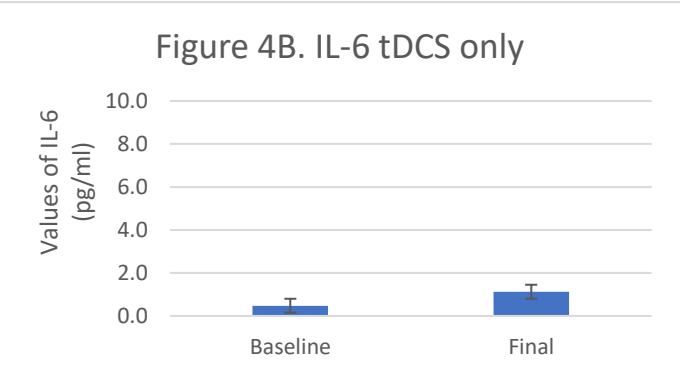
Time points	Sample number	Mean	SEM	*P* value
Baseline	9	7551.578	3886.1254	0.003
Midpoint	9	9645.299	6837.4341	0.003
Final	9	4610.965	4065.1777	0.003

Time points	Sample number	Mean	SEM	*P* value
Baseline	6	6459.623	3614.9118	0.082
Final	6	2959.998	3161.2828	0.082

Time points	Sample number	Mean	SEM	*P* value
Baseline	5	8825.540	5887.1980	0.019
Midpoint	5	4301.462	3874.5157	0.019
Final	5	4318.343	4158.2442	0.019

Tables are listed as n-3 PUFA treatment only with three timepoints, second table is tDCS only treatment with two timepoints, and the third table is the combination of n-3 PUFA and tDCS treatment with three time points of TNF- α. These tables show the repeated measure ANOVA results for TNF-α levels in all three groups. Originally, n-3 PUFA only group had 11, tDCS only group had nine, and n-3 PUFA and tDCS combination group had nine participants. Due to outliers in all groups, each groups' participant numbers decreased to run the ANOVA test. The differences in n-3 PUFA only group for TNF-α levels were significant ($p < 0.05$). tDCS only group TNF-α levels were not significantly different ($p < 0.05$). N-3 PUFA and tDCS combination group TNF-α levels were significant ($p < 0.05$).





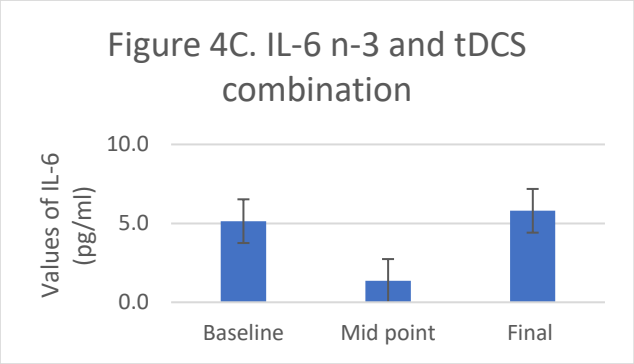


Figure 4 (A, B, and C). ±SEM IL-6 levels in baseline, midpoint, and final blood draws with n-3 PUFA ingestion. Note. Figure 4A shows the levels of IL-6 baseline, midpoint, and final blood draws for n-3 PUFA treatment. Figure 4B shows IL-6 levels at baseline and final blood draws with tDCS treatment. Figure 4C shows IL-6 levels in baseline, midpoint, and final blood draws with the combination of n-3 PUFA and tDCS treatment.

Figure 4A shows the effect of n-3 PUFA treatment for 6 weeks on plasma IL-6 levels. Between baseline and 4 weeks there was an apparent non-statistical 15% decrease in IL-6 levels. Between 4 weeks and 6 weeks of n-3 PUFA ingestion there was a doubling of IL-6 levels with a 68% increase from baseline to the final time point. tDCS only treatment for 4 weeks, five times a day for 30 minutes resulted in a more than doubling in IL-6 levels while in both cases, they remained significantly lower than the other treatment (see Figure 4B). With n-3 PUFA ingestion alone in the combination group, there was a 98% reduction in IL-6 that rebounded to marginally above baseline when combined with two weeks of tDCS therapy (see Figure 4C). As with TNF-α, IL-6 showed a different response in the two n-3 consuming groups where the n-3 only group had no change by midpoint while the combination group had

a significant reduction by this time. While both n-3 consuming groups had the same beginning point, where IL-6 doubled in 6 weeks with n-3 only, tDCS appeared to blunt this effect when combined with n-3 PUFA ingestion. This is interesting in that with tDCS alone, IL6 also doubled in 4 weeks. These data indicate that with sufficient n-3 PUFA ingestion for a long enough period of time or tDCS treatment, IL-6 increases and that with combination therapy of n-3 PUFA and tDCS, this effect is negated.

Table 3

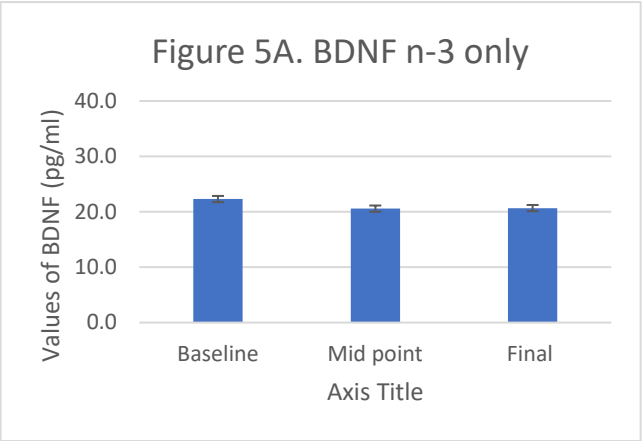
Statistical analysis of IL-6 levels in three groups

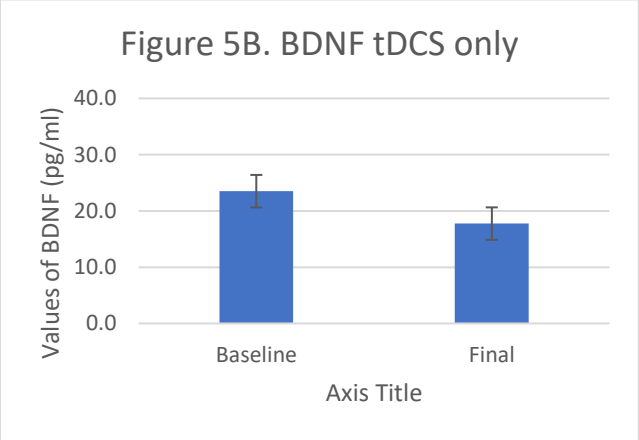
Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	513.938	897.9902	0.266
Midpoint	8	145.149	258.9555	0.266
Final	8	579.627	1019.3940	0.266

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	51.348	63.1757	0.232
Final	8	122.510	172.9784	0.232

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	513.938	897.9902	0.295
Midpoint	8	145.149	258.9555	0.295
Final	8	579.627	1019.3940	0.295

Tables are listed as n-3 PUFA treatment only with three timepoints, second table is tDCS only treatment with two timepoints, and the third table is the combination of n-3 PUFA and tDCS treatment with three time points of IL-6. These tables show the repeated measure ANOVA on IL-6 levels in all three groups. Due to having outliers in all of the three groups, the sample numbers decreased to eight for each group. N-3 PUFA group ($p < 0.05$), tDCS only group ($p < 0.05$), and n-3 PUFA and tDCS combination group ($p < 0.05$) show there is no significant change on IL-6 levels in all three groups.





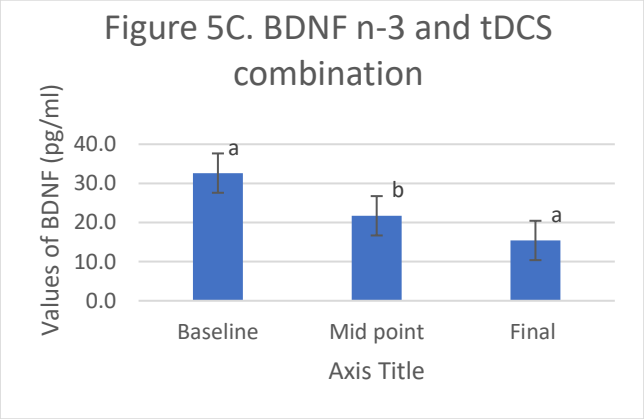


Figure 5 (A, B, and C). ±SEM BNDF with three different treatment groups. Note. Figure 5A shows BDNF levels at baseline, midpoint, and final blood draws. Figure 5B shows BDNF levels at baseline and final blood draws with tDCS treatment. Figure 5C shows BDNF levels at baseline, midpoint, and final blood draws with the combination of n-3 PUFA and tDCS treatments. (a and b in the figures stand for statistical difference).

With n-3 PUFA ingestion, there was a minimal 8% reduction in BDNF (NS) that did not differ between 4 and 6 weeks (see Figure 5A). With tDCS (see Figure 5B), while BDNF looked to have a greater reduction, this drop was again not significant. While combination treatment (see Figure 5C) appeared to reduce BDNF, due to large subject variations, this reduction was not significant (p <0.05). While n-3 PUFA appeared to reduce BDNF, and was further reduced with tDCS, neither timepoints varied from baseline. While baseline differed between the combination group and the other two groups, by midpoint there was no difference in the n-3 groups just as there was no difference in final numbers in the two tDCS groups. Combination group appears to have a greater impact on reducing BDNF than n3 PUFA or

tDCS alone. In the Figure 5C, the a and b letters represent the significant difference between the baseline and final time points (See table 8).

Table 4

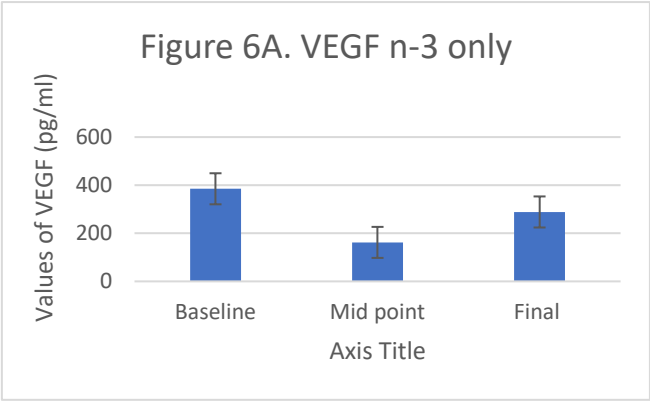
Statistical analysis of BDNF levels in three groups

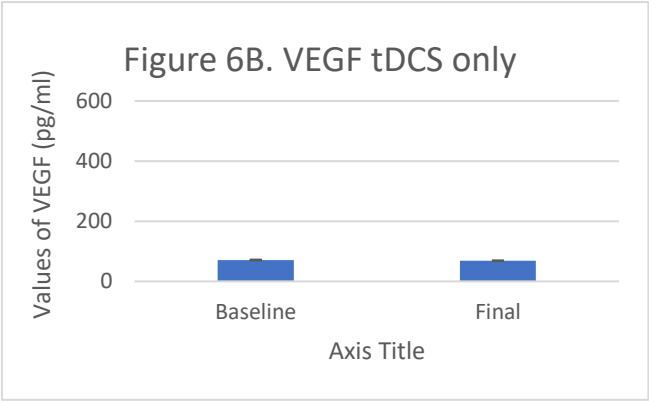
Time point	Sample number	Mean	SEM	*P* value
Baseline	10	19.599	15.8251	0.838
Midpoint	10	17.682	16.0787	0.838
Final	10	17.875	18.2055	0.838

Time point	Sample number	Mean	SEM	*P* value
Baseline	9	26.589	12.8182	0.346
Final	9	18.730	14.7644	0.346

Time point	Sample number	Mean	SEM	*P* value
Baseline	9	32.628	12.6667	0.091
Midpoint	9	21.717	11.5781	0.091
Final	9	15.412	15.9410	0.091

Tables are listed as n-3 PUFA treatment only with three timepoints, second table is tDCS only treatment with two timepoints, and the third table is the combination of n-3 PUFA and tDCS treatment with three time points of BDNF. These tables show the repeated measure ANOVA. Comparing the *p*-values between groups, n-3 PUFA $p > 0.838$, tDCS $p > 0.346$, and n-3 PUFA and tDCS combination $p > 0.091$ show that none of the treatment options had a significant effect on BDNF levels. This study had the outliers in BDNF levels in all three groups, and to run the ANOVA; the researcher had to and had to take out the outliers here as well.





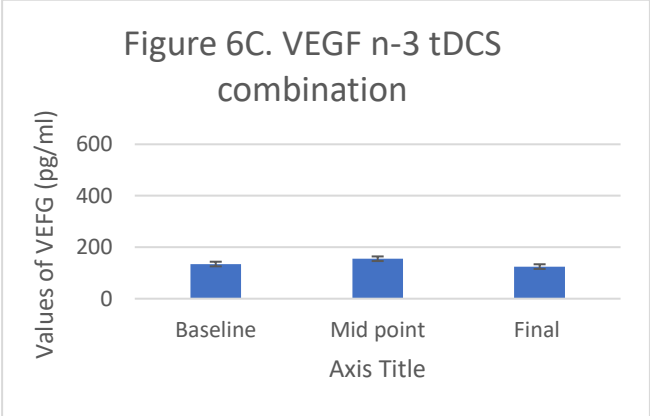


Figure 6 (A, B, and C). ±SEM VEGF with three different treatment groups. *Note.* Figure 6A shows VEGF levels in baseline, midpoint, and final blood draws with n-3 PUFA treatment. Figure 6B shows VEGF levels in baseline and final blood draws with tDCS treatment. Figure 6C shows VEGF levels in baseline, midpoint, and final blood draws with the combination of n-3 PUFA and tDCS treatment.

With n-3 PUFA consumption only, there was a 58% reduction in VEGF by 4 weeks that rebounded to a 25% reduction with 6 weeks of ingestion (see Figure 6A). With tDCS only, there was no change in VEGF levels (see Figure 6B). Unlike the n-3 PUFA only group where there was a non-statistical reduction in VEGF with 4-week n-3 PUFA intake, on the combination group, there was no change in VEGF with n-3 PUFA intake or combination treatment. While n-3 PUFA alone appear to induce non-statistical reductions in VEGF, when combined with tDCS, the effects appear to be negated. Significant variations in baseline levels may be hiding any biological impact that may have been recognized.

Table 5

Statistical analysis of VEGF levels in three groups

Time point	Sample number	Mean	SEM	*P* value
Baseline	9	134.697	115.0911	0.312
Midpoint	9	155.241	161.0313	0.312
Final	9	124.730	151.1282	0.312

Time point	Sample number	Mean	SEM	*P* value
Baseline	8	73.112	43.4834	0.945
Final	8	75.394	94.3350	0.945

Time point	Sample number	Mean	SEM	*P* value
Baseline	9	134.697	115.0911	0.441
Midpoint	9	155.241	161.0313	0.441
Final	9	124.730	151.1282	0.441

Tables are listed as n-3 PUFA treatment only with three timepoints, second table is tDCS only treatment with two timepoints, and the third table is the combination of n-3 PUFA and tDCS treatment with three time points of VEGF. These tables show the repeated measure ANOVA. In all three groups, the change within timepoints were not significant. VEGF n-3 PUFA treatment only *p*-value is $p > 0.312$, tDCS only treatment for VEGF is $p > 0.945$, and n-3 PUFA and tDCS combination treatment *p*-value is $p > 0.441$. Overall, in this study, only TNF-α had a significant change in response to n-3 PUFA only ($p < 0.003$) and n-3 PUFA and tDCS combination ($p < 0.019$) groups. For the most part, the other pro- and anti-inflammatory cytokines were not altered by treatment. While n-3 PUFA and tDCS alone or in concert did not alter IL-6 or VEGF, combining n-3 PUFA with tDCS resulted in an over 50% reduction in BDNF ($p < 0.05$), an effect that was not seen with the two treatment alone.

Table 6

Statistical analysis of TNF-α levels in baseline and final blood draws for three groups

Table 6.1.

TNF-α levels within baseline and final timepoints in n-3 PUFA only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	**9**	7551.57	3886.13	0.007
Final	**9**	4610.96	4065.17	0.007

Table 6.2.

TNF-α levels within baseline and final timepoints in tDCS only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	4773.776	4369.6028	0.099
Final	8	2236.305	2989.0541	0.099

Table 6.3.

TNF-α levels within baseline and final timepoints in n-3 PUFA and tDCS combination group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	9	11687.132	12319.3698	0.040
Final	9	4679.310	4634.5116	0.040

Note. Tables listed above represents the baseline and final timepoints of three groups of TNF-α levels. The first table represents the n-3 PUFA only group, second table represents tDCS only group, and the third group represents the n-3 PUFA and tDCS combination group. Comparing the baseline and final timepoints show that the n-3 PUFA only and the combination groups were significant within each group for TNF-α levels ($p < 0.05$).

Table 7

Statistical analysis of IL-6 levels in baseline and final blood draws for three groups

Table 7.1.

IL-6 levels within baseline and final timepoints in n-3 PUFA only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	11	492.489	800.5606	0.285
Final	11	827.880	1132.8531	0.285

Table 7.2.

IL-6 levels within baseline and final timepoints in tDCS only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	51.348	63.1757	0.232
Final	8	122.510	172.9784	0.232

Table 7.3.

IL-6 levels within baseline and final timepoints in n-3 PUFA and tDCS combination group.

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	513.938	897.9902	0.190
Final	8	579.627	1019.3940	0.190

Note. Tables listed above represents the baseline and final timepoints of three groups of IL-6 levels. The first table represents the n-3 PUFA only group, second table represents tDCS only group, and the third group represents the n-3 PUFA and tDCS combination group. Comparing baseline and final timepoints show that the two treatments were not significant ($p > 0.005$).

Table 8

Statistical analysis of BDNF levels in baseline and final blood draws for three groups

Table 8.1.

BDNF levels within baseline and final timepoints in n-3 PUFA only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	10	19.587	15.8303	0.671
Final	10	17.875	18.2055	0.671

Table 8.2.

BDNF levels within baseline and final timepoints in tDCS only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	24.668	14.9515	0.560
Final	8	18.815	15.3490	0.560

Table 8.3.

BDNF levels within baseline and final timepoints in n-3 PUFA and tDCS combination group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	9	32.628	12.6667	0.027
Final	9	15.400	15.9412	0.027

Note. Tables listed above represents the baseline and final timepoints of three groups of BDNF levels. The first table represents the n-3 PUFA only group, second table represents tDCS only group, and the third group represents the n-3 PUFA and tDCS combination group. Comparing two timepoints in all the groups show that combination group was significant ($p < 0.027$). The other two groups were not significant ($p > 0.05$).

Table 9

Statistical analysis of VEGF levels in baseline and final blood draws for three groups

Table 9.1.

VEGF levels within baseline and final timepoints in n-3 PUFA only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	11	384.993	1070.5945	0.644
Final	11	288.566	467.2198	0.644

Table 9.2.

VEGF levels within baseline and final timepoints in tDCS only group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	8	73.112	43.4834	0.945
Final	8	75.394	94.3350	0.945

Table 9.3.

VEGF levels within baseline and final timepoints in n-3 PUFA and tDCS combination group

Time point	**Sample number**	**Mean**	**SEM**	***P* value**
Baseline	9	134.697	115.0911	0.706
Final	9	124.730	151.1282	0.706

Note. Tables listed above represents the baseline and final timepoints of three groups of VEGF levels. The first table represents the n-3 PUFA only group, second table represents tDCS only group, and the third group represents the n-3 PUFA and tDCS combination group. Comparing two timepoints in all three groups show that none of the groups' VEGF levels were not significant ($p > 0.05$).

CHAPTER V

DISCUSSION

The goal of the Study

There are several studies that have identified possible treatment options for migraine and brain inflammation. Treatment options for migraines can be classified as pharmacological and non-pharmacological. While pharmacological treatment options for migraine have been increasing, typically most treatments are associated with increasing the dose levels of the pharmacologic agents (Ren et al., 2018). Increasing migraine medicine intake and dose is tied to long-term use of the medications, resulting in the need for higher doses to achieve the same functional effect in the body. Increased intake, frequency, or dose level a medication may result in a reduction in their functionality in the body and a lack of alleviation of a headache, or if it does have an effect, it does not relieve the headache entirely. The net effect is an increase in medication dose necessary to achieve the same desired endpoint. Increased medication dose and frequency is further complicated by side effects of the medications and could result in serious health problems such as gastrointestinal discomfort or stomach ulcers (Ren et al., 2018).

The researcher wanted aimed to investigate find a non-pharmacologic, cost-effective, and long-term potential treatment option for migraine while balancing cytokines associated with brain inflammation. N-3 PUFA may not only be a treatment option for migraines but would also have other health benefits including heart health. A portion of the original intent of the study was to evaluate the effect of these treatments on ceramide levels and composition; however due to construction in Old Main and chemical unavailability, the researcher will evaluate ceramide levels and composition prior to publication of this data.

Studies have evaluated the effect of n-3 fish oil and n-3 PUFA consumption on migraines or the alleviation of the headaches caused by migraine (Ren et al., 2018). This study evaluated the comparative effect of 2.2 to 2.4grams/day of long-chain n-3 PUFA for 6 weeks, and tDCS alone for 4 weeks or 2 weeks in concert with n-3 PUFA on cytokines that impact migraine. A majority of the tDCS only group participants suffered with migraines yet while participating in the study, they mentioned having no migraine events. Many recruits for the study, mentioned getting migraines during weather changes, finals week, and allergy seasons. While participating in the study, many of the participants mentioned not having any migraine episodes.

The cytokines evaluated in present study are known to function in the brain, with two being pro-inflammatory and two anti-inflammatory cytokines. Previous studies evaluating the effect of n-3 PUFA on the brain have focused on Alzheimer's disease, Schizophrenia, and inflammation (Hadjighassem et al., 2015). These diseases are related to an imbalance or changes in pro- and anti-inflammatory cytokine levels. BDNF is one of the most important cytokines in the brain, where it plays a key role in growth, maturation, and maintenance in the brain. It is an important mediator of neuroplasticity and is a positive contributor to neurogenesis. During inflammation, BDNF expression and function decreases and a reduction in neuroplasticity is seen. Studies that have examined BDNF and n-3 PUFA have been conducted in cell culture and human subjects using n-3 PUFA sources of fish and plant origin. The effectiveness of n-3 PUFA on BDNF and other cytokines is usually reflective of DHA and EPA levels in the study oils (Hadjighassem et al., 2015).

Dietary omega-3 fatty acids have a remarkable impact on the levels of DHA in the brain. Low levels of DHA in plasma can alter a neural development in children and cause dementia and cognitive decline in adults. The level of brain-derived neurotrophic factors

(BDNF) changes with dietary omega-3 fatty acid intake. BDNF is known for its effects in promoting neurogenesis and neuronal survival (Pawelcyzk, et al., 2019).

Other studies about n-3 PUFA and BDNF

Hadjighassem et.al (2015) assessed the effect of oral consumption of α-Linolenic acid (ALA) on blood levels of BDNF in healthy adult humans. Of 30 healthy volunteers, 15 men and 15 women, were selected randomly. Baseline and post 1-week flaxseed oil consumption plasma BDNF levels were assessed. Flaxseed was provided 500mg of alpha linolenic acid, daily for 1 week. It was revealed that plasma BDNF levels increased more in women than in men. Female BDNF levels increased by a 29% while male levels increased by 23%. The fact that BDNF increased with ALA ingestion indicated that ALA has therapeutic potential for the treatment of several neurological and psychiatric diseases. Considering the neuroprotective benefits of increased BDNF, ALA treatment could be a feasible approach to reduce brain issues in stroke patients (Pawelcyzk, et al., 2019).

N-3 PUFA may have the capacity to influence multiple biochemical mechanisms postulated to be involved in the pathogenesis of Schizophrenia that may be influenced by BDNF (Wu, et al., 2008). In a randomized placebo-controlled study Pawelczyk et al (2019) compared the efficacy of a 26-week intervention of either 2.2 g/day of n-3 PUFA or olive oil placebo on symptom severity in Schizophrenia patients. A secondary outcome measure was to identify any association between a n-3 PUFA clinical effect and changes in peripheral BDNF levels. There were 71 participants ages 16 to 35 enrolled in the study and randomly assigned to the EPA + DHA group or to the placebo group. Plasma BDNF was assessed at baseline, eighth and 26th weeks with BDNF level changes correlated with changes in symptom severity in different clinical domains. Significant increases in BDNF concentrations were observed in both groups during the study. The EPA + DHA group BDNF moved from 30pg/ml at the baseline to 49pg/ml at the 26th week, a 63% increase in BDNF levels. The placebo group saw

a 21% increase in BDNF from 33pg/ml at baseline to 40pg/ml at 26 weeks of olive oil ingestion. Further, the increase in BDNF showed a correlation with first-episode Schizophrenia an effect that may be triggered by the activation of intracellular signaling pathways including transcription factors such as cAMP-reactive element binding protein (Wu, et al., 2008).

N-3 PUFA and DHA effects on the brain are similar to exercise in how they impact and improve cognitive function, promote neuroplasticity, and protect against neurological lesions. A study by Wu et al. (2008) assessed the synergistic effect between DHA dietary supplementation and voluntary exercise on modulating synaptic plasticity and cognition in rats. Rats received DHA dietary supplementation (0.25% EPA and 1.25% DHA) or a placebo stock show diet with or without voluntary exercise for 12 days. Hippocampal BDNF levels were assessed following decapitation. The rats consuming the DHA-enriched diet performed better as evidenced by lower escape times than the rats maintained on the stock chow. Furthermore, results showed that exercise boosted the effect of DHA supplementation over DHA alone based on the latency escape time as an evaluation of escape exercise on cognitive function. While BDNF increased in rats fed the DHA diet by 138% and 128% in the exercised rats fed the stock diet, BDNF increased by 159% in exercising rats consuming the DHA diet. In rats, 12-day consumption of a DHA enriched diet significantly increased learning ability, with these effects enhanced by concurrent voluntary exercise. The effects of DHA and exercise on cognitive enhancement were paralleled by elevations in BDNF (Ahmadi, et al., 2014)

This study provided 2,400mg of mixed n-3 fatty acids, 1272 to 1440mg EPA and 816 to 960mg DHA daily for 6 weeks. Comparatively, this study provided marginally more n-3 PUFA as EPA, and DHA, but was 75% shorter in duration than the study by Pawelczyk et al. (2019), who found a 63% increase in BDNF between baseline and the final time points.

The Pawelczyk et al. study indicates that longer duration n-3 PUFA intake is more effective at increasing the BDNF levels. In this thesis study, there was no change in BDNF at both midpoint and by the 6-week end of the study. This may be reflective of the need for a longer n-3 PUFA ingestion period to induce a significant biological change. In the mix gender study by Hadjighassem et al. (2015), plant-based n-3 PUFA increased BDNF levels within a week by 23 to 29%; comparatively, in this thesis study BNDF was reduced by n-3 PUFA, tDCS and n-3 PUFA and tDCS combined. The differences exhibited in this thesis study versus others may be attributable to a smaller sample size, though the direction of change is surprising. The interesting finding is that tDCS and n-3 PUFA combined appeared to have an additive effect on reducing BDNF, an effect the opposite of what was anticipated.

Other studies about n-3 PUFA and VEGF

VEGF stimulates the formation of blood vessels by a process called angiogenesis. During inflammation, elevated VEGF can be dangerous attributable to its promotion of angiogenesis. In healthy individuals, high or increasing levels of VEGF are viewed as beneficial. In inflammation or many chronic diseases, decreased VEGF is desired to prevent the formation of new blood vessels.

The n-3 PUFA, EPA, and DHA are known for their beneficial effect in brain health, growth and development, and cardiovascular disease. Their benefit is believed to be related to their anti-inflammatory and antioxidant capability. EPA and DHA can inhibit inflammatory cytokine-induced endothelial cell activation and reduce endothelial migration and proliferation. In a study by Ahmadi et al (2014), the effect of vitamin E and n-3 PUFA on endothelial function in adolescents with metabolic syndrome was examined. Ninety young individuals aged 10 to 18 years with metabolic syndrome were randomly assigned to receive either vitamin E tablets (400 IU/day) or n-3 PUFA tablets (2.4 g/day) or placebo. N-3 ingestion for 8 weeks led to a significant increase in serum HDL levels when compared to the

vitamin E or placebo treatment group. With n-3 PUFA ingestion, there was a 51% reduction in VEGF from 211.96pg/dL to 103.95pg/dL, while in the vitamin E group VEGF was only reduced by 10% from 249.93pg/dL to 227.05pg/dL (Jamilian, et al., 2018).

Jamilian et al. (2018) examined the influence of vitamin D and n-3 PUFA supplementation on clinical, metabolic, and genetic parameters in women with polycystic ovary syndrome (PCOS). Polycystic ovaries upregulate VEGF associated with an increased vascularity. Sixty subjects 18 to 40 years of age with PCOS were randomized to consume either vitamin D (50,000 IU biweekly) plus 2 g/day n-3 PUFA from fish oil or placebo for 12 weeks. Vitamin D and n-3 PUFA supplementation significantly upregulated VEGF levels of women with PCOS ($p < 0.004$). Further, co-administration of vitamin D and n-3 PUFA improved total antioxidant capacity and reduced inflammation in the body (Moghadam, et al., 2012).

In this thesis study, no participants appeared to start with any inflammation, thus we anticipated only moderate VEGF levels prior to the study. In the n-3 PUFA only and combination groups, when the VEGF levels were combined and evaluated, VEGF levels by midpoint were reduced. Further, in the combination group, VEGF remained unchanged from the midpoint levels with the addition of tDCS. This thesis study and Ahmadi et al.'s (2014) study provided the same n-3 PUFA dosage (2.4g/day), while 2g/day was provided in the study of Jamilian et al. [92]. Comparatively, this thesis study was 6 weeks long, the Ahmadi (2014) study was 8 weeks long, and Jamilian's study (2018) was 12 weeks long. In agreement with the others, this thesis study also showed a reduction in VEGF by midpoint to 6-weeks, though there was a partial rebounded in the final weeks of our study. By the midpoint, the combined n-3 PUFA and combination groups, VEGF levels were reduced by 42%, a common treatment for both groups, with continued n-3 only ingestion group, VEGF remained 46% while in the combination group VEGF was the same as baseline. By comparison, tDCS did not affect

VEGF, but when combined with n-3 PUFA, appears to negate the changes induced by n-3 PUFA alone. While n-3 PUFA alone appeared to reduce VEGF, Combined n-3 PUFA and tDCS would be in effective in altering VEGF levels. Though tDCS effect might be attributable to the short duration of the study, n-3 PUFA reduced VEGF, an effect that was negated with tDCS therapy.

Other studies about n-3 PUFA and TNF-α

TNF-α is increased in serum and tissues during inflammation and infectious conditions. During inflammation, TNF-α can induce an increase in the severity of the disease by promoting to apoptosis. Moghadam et al. (2012). aimed to determine the effect of n-3 PUFA supplementation on the serum levels of TNF-α in type 2 diabetes mellitus (T2DM) patients. In the study, 84 subjects aged 45–85 years with at least a 2 year history of T2DM either received three n-3 capsules per day (EPA 1,548 mg; DHA 828 mg; other n-3 fatty acids 338 mg), or three placebo capsules (sunflower oil 2,100 mg)as a control for a period of 8 weeks. At the end of the study, n-3 PUFA supplementation led to decreased serum levels of TNF-α concentration by 8% in the treatment group ($p < 0.01$; Holm, et al., 2001).

In a study by Holm et al., (2001), TNF-α in heart transplant recipients was examined. The patients were long-time survivors of heart transplantation and received n-3 fatty acids (3.4g/day) or placebo for a year. As expected, tissue EPA and DHA levels increased significantly over time in the n-3 group but not in the placebo group. There was a significant correlation between changes in levels of TNF-α and EPA with a 16% increase in TNF-α in a year (Tayyebi-Khosroshahi, et al., 2012).

Tayyebi-Khosroshahi (2012) assessed the effect of n-3 PUFA on TNF-α levels and chronic kidney failure and C-reactive protein (CRP), a strong independent risk marker of cardiovascular disease (CVD). The aim of this study was to examine correlation between serum levels of TNF-α, CRP and other parameters induced by n-3 supplementation in 37

patients with end-stage renal disease undergoing dialysis. The patients received 3g/day n-3 for 2 months. Average TNF-α levels of 6.91pg/ml were reduced to 2.35pg/ml (65% decrease) with n-3 PUFA supplementation. N-3 PUFA supplementation decreased not only TNF-α but also CRP inducing a cardioprotective benefit. At the molecular level, n-3 PUFA appears to act by reducing leukocytosis, systemic inflammation, and oxidative stress (Kiecolt, et al., 2012).

This thesis study provided 1272-1440mg EPA and 816-960mg DHA daily for 6 weeks and resulted in a 45% reduction in TNF-α in this time period ($p < 0.003$). Moghadam et al. (2012) provided 1.54mg EPA and 828mg of DHA for 8 weeks and induced an 8% decrease in TNF-α levels. Comparatively, Holm, et al., (2001) found yearlong study providing 3.4g/day of n-3 PUFA induced in a 16% increase in TNF-α levels. This relationship is likely how n-3 PUFA induce an immunologic benefit. Tayyebi et al. (2012) found that providing 3g/day of n-3 PUFA for 2 months reduced TNF-α by 65%. Tayyebi et al. (2012), also found that the cell membrane fatty acids played an important role in signal transduction where the n-3 PUFAs were able to modify gene expression. By mediating cell membrane function and structure and the synthesis of the eicosanoids, n-3 PUFA may cause multiple health benefits in dialysis patients.

Other studies about n-3 PUFA and IL-6

IL-6 is a cytokine involved in the immune response in healthy people. It can act as a pro- or anti-inflammatory cytokine. During inflammation, increased IL-6 can increase the severity of a disease response [54]. Observational studies have linked lower levels of n-3 PUFA with inflammation and depression. In a study by Kiecolt-Glaser et al. (2012), the effect of n-3 supplementation on serum cytokine production and depressive symptoms in 138 healthy middle-aged (40 to 85 years old) and older adults was evaluated. A 4-month trial evaluated (1) 2.5 g/d n-3 PUFAs, or (2) 1.25 g/d n-3 PUFAs, or (3) placebo capsules that mirrored the fatty acids proportions in the typical American diet. In the higher n-3 group, IL-6

level decreased 10%, while in the lower n-3 group, IL-6 levels decreased 12%. Significant differences were observed between both n-3 groups and the placebo group. Importantly, where IL-6 decreased in response to n-3 PUFA, it increased by 36% in the placebo group (Kiecolt-Glaser, et al., 2011).

Another study of Kiecolt-Glaser et al. (2011), examined the effects of n-3 PUFA on inflammation, anxiety, and IL-6 was assessed in 68 medical students in a 12- week long study using either 2.5 g/d n-3 PUFA (2085 mg EPA and 348 mg DHA) or a placebo that mirrored the fatty acids proportions in the typical American diet. Students receiving the n-3 PUFAs had a 14% reduction in IL-6 production and a 20% reduction in anxiety symptoms compared to controls (Przeklasa, et al., 2017).

In this thesis study, where the provided 2,400mg of mixed n-3 fatty acids for 6 weeks, Kiecolt-Glasser (2011) provided either 1.25g/day or 2.5g/day, where Kiecolt-Glasser found IL-6 reduction with n-3 PUFA ingestion in both of their studies. We had increase in our IL6 levels in all three groups. In the anxiety study, Kiecolt-Glasser found a significant effect of n-6: n-3 ratio where, if the intake of n-6 increases, IL-6 production increases and may lead to increase in inflammatory-related diseases. As mentioned earlier, in this thesis study, while participants did not have an inflammation, the researcher did not track participant food intake. If participants consumed more n-6 than n-3 PUFA, it may have confounded the results and influenced the outcomes.

tDCS is known to induce long-lasting alterations of cortical excitability both in experimental animals and humans. Recent studies have tried to exploit the therapeutic potential of the method for various neurological and psychiatric disorders. Studies in human stroke patients have shown tDCS to be a promising therapeutic intervention to ameliorate motor deficits [84]. Neurological symptom evaluated the impact of the tDCS on the consumption of drugs and on pain conditions (frequency, duration, intensity). In a study by

Przeklasa-Muszyńskaet al. (2017), 50 patients with migraine headache (30 with aura, 20 without aura) were evaluated. In 30 patients (18 with aura, 12 without aura) previous unsatisfactory treatment was supplemented with tDCS; and 20 patients (12 with aura, 8 without aura); from a control group were treated by pharmacological methods. Observations continued for 30 days after the stimulation at which time it was reported that tDCS treatment lead to a reduction in the consumption of analgesics and triptans. Additionally, pain intensity decreased during pain episodes, along with a reduction in duration of episodes and the number of pain days. The subjective assessment of pain reduction indicated that in migraine patients, there was a 36 to 40% reduction after tDCS an effect that was much more effective in comparison to the group receiving pharmacotherapy (10 to 12.5%). The Przeklasa-Muszyńska et al. (2017), results suggest that tDCS may be a safe and useful clinical tool in migraine prophylaxis and treatment (Przeklasa, et al., 2017).

A majority of the studies related to the cytokines and tDCS are systematic reviews of the function of the cytokines and tDCS, looking at the relationship between tDCS and cytokines. This thesis study's results show that tDCS decreased TNF-α levels in tDCS only group and last 2 weeks of the combination group. The effect of tDCS in the combination group was statistically significant. IL-6 levels with tDCS treatment increased only in the tDCS combination group. tDCS also decreased the BDNF and VEGF levels. In the VEGF combination group, the combination with n-3 PUFA, the reduction was slightly lower than the midpoint.

Comparing this study with other studies where the same cytokines were assessed, increases and decreases were not the same, an effect that may be attributable to differences in age, gender, dosage difference, time length of the study, and potential combinations of different vitamins other treatment options. While participants did not have brain inflammation, some of them reported having migraine. As participants did not have chronic

disease and were healthy individuals, the cytokine data would differ from those studies where participants had inflammation present.

Limitations

Limitations in the study may include high concentrations of metabolites in the plasma for BDNF analysis, it was necessary to dilute the samples on the ELISA plate, an effect that may not truly reflect BDNF present. The study was conducted near the end of the spring 2019 semester during one of the worst allergy seasons in Texas. Due to the potential of chronic inflammation, this must be taken in evaluating these finding.

Future research

The length of the study needs to be longer to receive more reliable results on cytokines levels. Also, diluting the samples before running the EILSA plates will save time and give accurate results.

Conclusion

The purpose of the study was to find a low cost and cost-effective treatment option for migraine and brain inflammation. N-3 and tDCS have been studied separately and combined for both migraine and brain inflammation. While induced changes where not recognized in all cytokines, the changes in VEGF and TNF-α do indicate that n-3 PUFA may ameliorate inflammation. The bigger issue may be the effect of tDCS where it eliminated some of the beneficial increase in VEGF induced by n-3 PUFA ingestion. While cytokine profiles were not considerably altered, participants provided positive feedback during and after the study. They have mentioned a reduction in migraines and better focus on their work and studying resulting in better relations with their spouse, kids, families, and friends.

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APPENDIX A

Texas Woman's University Institutional Review Board Human Studies Subjects Review Committee Approval

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619 940-
8983378 email: IRB@twu.edu
https://www.twu.edu/institutional-review-boardirb/

DATE: March 14, 2019

TO: Ms. Ezgi Ozturk
Nutrition and Food Sciences

FROM: Institutional Review Board - Denton

Re: Notification of Approval for Modification for Effects of Omega-3 Fatty Acid Supplementation and tDCS on Plasma Ceramide Levels and Ceramide Composition (Protocol #: 20187)

The following modification(s) have been approved by the IRB:

Kaylie Daniels, the original PI of the study is no longer working on the study and has been removed from the research team. Ezgi Ozturk will now be the PI for the study. All recruitment material and consent form have been updated to reflect this change. cc. Dr. Shane Broughton, Nutrition and Food Sciences

APPENDIX B

Texas Woman's University Institutional Review Board Human Studies Subjects Review Committee Approval

Institutional Review Board
Office of Research and Sponsored Programs
P.O. Box 425619, Denton, TX 76204-5619 940898-3378
email: IRB@twu.edu
https://www.twu.edu/institutional-review-board-irb/

DATE: September 16, 2019
TO: Ms. Ezgi Ozturk
Nutrition and Food Sciences
FROM: Institutional Review Board (IRB) - Denton

Re: Extension for Effects of Omega-3 Fatty Acid Supplementation and tDCS on Plasma Ceramide Levels and Ceramide Composition (Protocol #: 20187)

The request for an extension of the IRB approval for the above referenced study has been reviewed by the TWU IRB (operating under FWA00000178). This study was originally approved on September 7, 2018 and has been renewed. Approval for this study expires on September 6, 2020.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. If subject recruitment is on-going, a copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any unanticipated incidents. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc.Dr. Shane Broughton, Nutrition and Food Sciences

APPENDIX C

Consent form

TEXAS WOMAN'S UNIVERSITY
CONSENT TO PARTICIPATE IN RESEARCH

Title of Study: **Control Population for Firefighter Study**

Investigator's Name: Shane Broughton, PhD

Department of Nutrition and Food Sciences

Investigator's Phone: (940) 898-3715

Investigator's Email: kbroughton@mail.twu.edu

Co-Investigator's Name: Jyutika Mehta, PhD
Department of Communication Sciences and Oral Health
Co-Investigator's Phone (940) 898-2029
Co-Investigator's Email: jmehta@twu.edu

Key Information

The purpose of these **tests** is to compare possible indicators of inflammation in Denton Firefighters to a non-firefighter control population. This data collected in the Pioneer Performance Clinic will be used for research purposes. You will only be required to provide a single blood sample to investigate the association between stress and brain plasticity and should only require 5-10 minutes of time.

Research Procedures

Blood collection: You will not be required to fast for these draws. A single venous blood sample will be drawn by a trained phlebotomist using aseptic, standard procedures. A single blood sample will be drawn directly from the antecubital vein into a sterile 6.0 ml sodium heparin vacutainer tube and centrifuged at 3000 rpm for 15 min at 4˚C. Heparinized plasma samples thus obtained will be stored at -70°C in microcentrifuge tubes (at WH 017) until analyses. These samples will be used for the biochemical quantification of mediators of neuroplasticity, namely vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF) and C-reactive protein (CRP). The blood isolation can be completed in approximately 10 minutes.

Potential Risks

Loss of Confidentiality: There exists the possibility of the loss of confidentiality as a potential risk of participation in this study. Confidentiality will be protected to the extent that is allowed by law. To minimize this risk, all data will be kept in a locked file cabinet in Pioneer Performance Clinic (PPC), 011 Woodcock Hall, Texas Woman's University. Data will only include age and gender associated with the sample to allow for gender and age match to the firefighter population. A single identification form will be used to link age and gender to a numerical code. This will be the only way to connect data with demographic information. This form will be kept in a separate file than all other data in the PPC.

Participant Initials _____

Bruising: The risk of bruising resulting from blood draws is minimal due to this procedure being performed by trained personnel. Universal precautions will be used during all blood draw procedures. To minimize bruising, pressure will be applied to the site for approximately five minutes after the blood draw.

Infection: The risk of infection resulting from blood draws is minimal due to the procedures being performed by trained personnel. Universal precautions will be used during the blood draw. The site for blood draw will be cleaned with alcohol immediately prior to venipuncture. Each new needle that is opened will be disposed of in biohazard boxes immediately after use.

Latex Allergy: The phlebotomist will typically wear nitrile gloves during all blood draws. Prior to the blood draw, you will be asked if you are allergic to latex. If you inform the phlebotomist that you are allergic to latex, a nitrile glove and tourniquet will be used.

Participation and Benefits

Participation in this study is voluntary and as a participant, you have the right to withdraw from the study at any time without penalty. Should you desire to withdraw from the study at any point, you are entitled to be informed of any data collected from you that has been analyzed at any time point.

All identifiers will be removed from the identifiable private information or identifiable biospecimens and, after such removal, the information or biospecimens may be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or the legally authorized representative. All unidentifiable data will be kept indefinitely for future research projects. If you would like to participate in the current study but not allow your de-identified data to be used for future research, please initial here _____.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Questions Regarding the Study

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you should ask the researchers. If you have any questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at (940) 898-3378 or via email at IRB@twu.edu.

_____ _____

Signature of Participant Date

Approved by the
Texas Woman's University
Institutional Review Board

Approved: March 12, 2019

Modifications Approved:

June 4, 2019

APPENDIX D

Recruitment flier

TEXAS WOMAN'S UNIVERSITY

VOLUNTEERS NEEDED FOR RESEARCH STUDY

Effects of Omega-3 Fatty Acid Supplementation and Transcranial Direct Current Stimulation on Plasma Ceramide Levels

To Participate:

Must be a healthy female between 18-45 years old and living in the Denton area

Must be willing to take either fish oil supplements and/or receive non-invasive brain stimulation

Details:

Will receive free supplements or stimulation sessions

Study length is maximum of 6 weeks

Urine and blood samples will be collected maximum of 3 times

If you are eligible and interested or if you have questions regarding eligibility contact:

Ezgi Ozturk

eozturk@twu.edu

Confidentiality Statement: There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions. This study is voluntary and you may discontinue at any time.

APPENDIX E

Health History questionnaire

Health Questionnaree

Name _____

Date

____/____/20_____ (Last) (First) (Middle)

Circle One	**Notes**
(1) the care of a physician during the past 2 years?	**Have you been under Yes No**
(2) penicillin, any drugs, medicine, or latex?	**Are you allergic to Yes No**
(3) excessive bleeding which required special treatment?	**Have you ever had Yes No**
(4) chance you might be pregnant?	**Women: Is there a Yes No**
(5) taking any birth control medication?	**Women: Are you Yes No**
(6) adverse reaction to local anesthetics?	**Have you had Yes No**

(7) Do you use recreational drugs?_____ If yes, what?_____

(8) Do you use tobacco? _____ If so, what form? _____

(9) Date of last medical exam _____

(10) Circle Yes to any of the following which you have had or have at present. Circle No to those that you have not had.

Yes No **High Blood Pressure**
Yes No **High Blood Cholesterol**
Yes No **Chest Pain or Pressure (Angina)**
Yes No **Heart Disease or Attack**
Yes No **Heart Pacemaker**
Yes No **Heart Failure**
Yes No **Heart Surgery**
Yes No **Fainting or Lightheadedness**
Yes No **Artificial Heart Valve**
Yes No **Congenital Heart Lesions**
Yes No **Mitral Valve Prolapse**
Yes No **Stroke**
Yes No **Transient Ishemic Attack**
Yes No **Lupus**
Yes No **Rheumatic Fever**
Yes No **Scarlet Fever**
Yes No **Chronic Fatigue**
Yes No **Artificial Joints**
Yes No **Kidney Dialysis**
Yes No **Kidney Disease**
Yes No **Eating Disorder**
Yes No **Rheumatoid Arthritis**
Yes No **Arthritis**
Yes No **Chronic Head, Neck, or Back Pain**
Yes No **Diabetes Requiring Insulin**
Yes No **Diabetes Not Requiring Insulin**
Yes No **Hypoglycemia**
Yes No **Hyperthyroidism (High)**
Yes No **Hypothyroidism (Low)**
Yes No **Ulcers**
Yes No **Pulmonary Disease**
Yes No **Unexplained Shortness of Breath**
Yes No **Chronic Cough or Bronchitis**
Yes No **Tuberculosis (TB)**
Yes No **Emphysema**
Yes No **Asthma**
Yes No **Hay Fever**
Yes No **Allergies or Hives**
Yes No **Sinus Trouble**
Yes No **Cancer**
Yes No **Leukemia or Lymphoma**
Yes No **Radiation or Chemotherapy**
Yes No **Anemia**
Yes No **Bruise Easily**
Yes No **Bleeding Disorders**
Yes No **Sickle Cell Disease**
Yes No **Alcoholism**
Yes No **Drug Addiction**
Yes No **Blood Transfusion**
Yes No **Liver Disease**
Yes No **Yellow Jaundice**
Yes No **Hepatitis**

Yes No **Psychiatric Treatment**
Yes No **Depression / Bipolar**
Yes No **Nervousness / Anxiety**
Yes No **Dizzy Spells**
Yes No **Epilepsy or Seizures**
Yes No **Condition Requiring Medicine Cortisone**
Yes No **Glaucoma**
Yes No **Spine or Hip Fractures**

Yes No AIDS / HIV Infection
Yes No Cold Sores / Fever Blisters

List all prescription medications that you are currently taking.

Medication/Dosage/Date Started/Reason

_____

Medication/Dosage/Date Started/Reason

_____

Medication/Dosage/Date Started/Reason

_____

Medication/Dosage/Date Started/Reason

_____

Medication/Dosage/Date Started/Reason

_____

Medication/Dosage/Date Started/Reason

_____

Please list all non-prescription medication or vitamins or nutritional supplements you are currently taking.

Name/Dosage/Date Started/Reason

_____ _____

Name/Dosage/Date Started/Reason

_____ _____

Name/Dosage/Date Started/Reason

_____ _____

Name/Dosage/Date Started/Reason

_____ _____

Name/Dosage/Date Started/Reason

_____ _____

Name/Dosage/Date Started/Reason

_____ _____

List all surgical procedures that you have had in the past.

Year _____ Type of Surgery/Reason

_____

Year _____ Type of Surgery/Reason

_____

Year _____ Type of Surgery/Reason

_____

Year _____ Type of Surgery/Reason

_____

List all hospitalizations of 24 hours or more for any reason.

Year _____ Reason for hospitalization

_____

Year _____ Reason for hospitalization

_____

Year _____ Reason for hospitalization

_____

Year _____ Reason for hospitalization

_____

Other Health Information

Please use this space to record any other personal health information that was not listed above.

_____
_____

_____
_____

"I Attest To The Fact That The Information Given Above Is Correct."

_____
(Parent or Guardian must sign for patient under age 18.)

This section for office use only:

Ht:

Wt:

BMI:

Comments:

_____
_____
_____
_____
_____
_____
_____
_____

APPENDIX F

Diet history questionnaire

Diet History Questionnaire

Number of meals you usually eat per day: _____

Number of times per week you usually eat the following:

Beef _____ Fish _____ Desserts _____

Pork _____ Fowl _____ Fried Foods_____

Number of servings (cups, glasses, or containers) per week you usually consume of:

Milk _____ Coffee _____

Tea (iced or not) _____ Regular or diet sodas _____ Glasses of water_____

Do you ever drink alcoholic beverages?

☐ Yes ☐ No

If yes, what is your approximate intake of these beverages?

Beer:

☐ None ____ per week

Wine:

☐ None ____ per week

Hard Liquor:

☐ None ____ per week

At any time in the past, were you a heavy drinker (consumption of six ounces of hard liquor per day or more)?

☐ Yes ☐ No

Do you usually use oil or margarine in place of high cholesterol shortening or butter?

☐ Yes ☐ No If yes, which oils _____

Do you usually abstain from extra sugar usage?

☐ Yes ☐ No

Do you usually add salt at the table?

☐ Yes ☐ No

Do you eat differently on weekends as compared to weekdays?

☐Yes ☐No

Are you taking any vitamin/mineral/herbal supplements?

☐Yes ☐No If yes, please list: _____

Additional Comments:

_____

_____

APPENDIX G

Migraine/Headache questionnaire

Migraine questionnaire

1. Do you have migraines/headaches? If yes, what is the frequency and duration in the past month?

   _____

   _____

   _____

2. In the past month how would you describe your ability to concentrate or pay attention?

   _____

   _____

   Additional Comments:

   _____

   _____

   _____

   _____ ___