Diet, Inflammation, Gut Microbiome, and Mental Health

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DIET, INFLAMMATION, GUT MICROBIOME, AND MENTAL HEALTH

by

ASHLEY ROSE POLOKOWSKI

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2019
Diet, Inflammation, Gut Microbiome, and Mental Health

by

Ashley Rose Polokowski

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Omega-3 fatty acids (ω-3 FAs) are an essential fatty acid necessary for healthy development in mammals. They possess anti-inflammatory properties and have more recently been shown to impact gut microbiota, both factors hypothesized to be associated with depression and anxiety. Thus, empirical efforts have begun to examine the benefit of ω-3 FAs as a treatment option for various psychological disorders. Although there is evidence that ω-3 FAs have favorable outcomes on depressive symptoms, the relationship between ω-3 FAs and anxiety and the pathways by which ω-3 FAs produce beneficial health effects are poorly understood. Both inflammation and the gut microbiome have an impact in the development of anxiety and depression, thus these factors may play a fundamental role as mechanisms explaining the beneficial effect of ω-3 FAs on psychological health. This study aims to 1) to examine the cross-sectional relationship between ω-3 FAs; gut microbiome bacteria and inflammatory markers; and stress, anxiety, and depressive symptoms; and 2) to examine if the gut microbiome and inflammatory markers mediate the relationship between ω-3 intake and stress, anxiety and depressive symptoms. Results indicate that higher levels of omega-3 index and DHA were associated with lower reported trait-anxiety symptoms, depressive symptoms, and lower concentrations of the inflammatory marker IL-1β. There was no evidence that gut bacteria or
inflammation mediated the relationship between ω-3 FAs and stress, anxiety, or depression symptoms, but several significant associations were noted. In particular, findings suggest that increased omega-3 index and DHA levels predicted fewer trait-anxiety and depression symptoms. Additional research is warranted to elucidate the interplay of mechanisms that may explain the impact of ω-3 FAs on mental health outcomes.
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CHAPTER 1: INTRODUCTION

Diet and Mental Health

One of the most under-recognized factors in the development of mental health disorders is the role of nutrition. It is well established that unhealthy dietary habits contribute to negative physical health consequences worldwide, and it is estimated that the U.S. Department of Agriculture spends millions of dollars annually on nutritional education programs (e.g., The Expanded Food and Nutrition Education Program) to improve dietary habits (United States Department of Agriculture, 2016).

In addition to the associated physiological health consequences, accumulating evidence supports that diet can play a role in the development, management and prevention of various mental health problems such as anxiety and depression. A global meta-analysis showed that higher consumption of fruits, vegetables, fish, and grains were associated with lower odds of developing depression in adults (Lai et al., 2013). This effect was also found in older adults (≥60 years) in a cross-sectional study comparing clinically depressed and non-depressed older adults (≥60 years) (Payne, Steck, George, & Steffens, 2012).

The impact of diet on psychological health has also started to be explored in child and adolescent populations. A systematic review found a significant association between unhealthy diet and poorer mental health outcomes (e.g., depression, anxiety) across seven countries around the world (O’Neil et al., 2014). In a recent cross-sectional study of Iranian adolescent girls, those whom consumed more pro-inflammatory diets (e.g., high in carbohydrates and unhealthy fats) had greater levels of stress (Shivappa, Hebert, & Rashidkhani, 2017).

Aside from anxiety and depressive symptoms, diet has also been shown to affect other dimensions of mental health such as neuroplasticity (Murphy et al., 2014), protection against
cognitive decline (Naqvi et al., 2011), development of subcortical brain structures, and verbal IQ (Isaacs et al., 2008). Collectively, these associations have led researchers to investigate the role of nutrition and nutritional supplements on treating mental health disorders including depression and anxiety.

Omega-3 Fatty Acids (ω-3 FAs)

Omega-3 fatty acids are acknowledged as a necessary component of a healthy lifestyle because they possess numerous physical and mental health benefits (Ruxton, Reed, Simpson, & Millington, 2007).

Omega-3 fatty acids (referred to as ω-3 FAs) and omega-6 fatty acids (referred to as ω-6 FAs), are two major classes of long chain polyunsaturated fatty acids (PUFA) that contain two or more double bonds. Each of these PUFAs are essential fatty acids (EFA), meaning they cannot be synthesized by mammals and must be obtained by the diet but are physiologically necessary for normal mammalian cell functioning (Innis, 1991), including central nervous system development (Perica & Delaš, 2011). The essential fatty acid alpha-linolenic acid (ALA) is the precursor to the ω-3 FAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The essential fatty acid linoleic acid (LA) is the precursor to the ω-6 FA arachidonic acid (AA). A balance of ω-3 and ω-6 FAs are necessary for proper health and development (Simopoulos, 2002).

The three main ω-3 FAs important to human functioning are: ALA with an 18-carbon chain, EPA with a 20-carbon chain, and DHA with a 22-carbon chain. Each of the ω-3 FAs can be obtained from dietary sources, ALA from plant-based oils (e.g., seeds, nuts) and EPA and DHA from marine based oils (e.g., fish, algae). In addition to obtaining it from dietary sources, humans can convert the essential fatty acid, ALA, into the longer chain ω-3s, EPA and DHA
although this conversion is inefficient (Anderson & Ma, 2009; Burdge & Calder, 2005). Within the brain of mammals, DHA is the most common ω-3 FA and plays a critical role in the development and function of the brain throughout the life span (Innis, 2007). EPA and DHA have both been deemed necessary for healthy brain function and retina development, are a protective factor against coronary heart disease and diabetes, and are associated with reduced cancer growths (Narayan, Miyashita, & Hosakawa, 2006).

Over the past century, there has been a drastic shift in dietary patterns, including the substantially increased consumption of ω-6 fatty acids, due to the large amount of linoleic acid-enriched vegetable oils in the food supply, and subsequent deficiency of ω-3 FAs in the Western diet (Simopoulos, 2002). The consumption of excessive amounts of ω-6 FAs paired with depleted levels of ω-3 FAs in many human diets foster the development of numerous diseases such as inflammatory diseases, cancer, and cardiovascular disease (Simopoulos, 2001). In a study analyzing risk assessment of dietary risk factors in preventable causes of death in the United States, results demonstrate that high dietary trans-fatty acid content, high dietary salt content, and low ω-3 FA content were the dietary risks found to have the largest mortality effects (Danaei et al., 2009).

Omega-3 FAs show such promise in improving health that they are being used as a treatment option for various inflammatory diseases such as rheumatoid arthritis (James & Cleland, 1997), Crohn’s disease (Belluzzi et al., 1996), and asthma (Broughton, Johnson, Pace, Liebman, & Kleppinger, 1997). Furthermore, preclinical research has noted a fundamental role of ω-3 FAs within brain functioning and mental health, in which a lack of these fatty acids in the brain can stimulate anxious and depressive behaviors (Müller et al., 2015). Although the literature has begun to acknowledge the positive impact of ω-3 FAs on chronic mental health
conditions like depression and anxiety, the pathways by which ω-3 FAs demonstrate beneficial effects on mental health is not as well-defined.

**ω-3 FAs and Depression**

A growing literature suggests that ω-3 FA supplementation has therapeutic benefits in reducing depressive symptoms. Early findings in rodents indicate that ω-3 FAs play a favorable role on depressive behaviors. Rats fed a diet deficient in ω-3 FAs, spent significantly more time immobile on the forced swim test for depression compared to rats fed ω-3 adequate diets (DeMar et al., 2006; Levant et al., 2008). Furthermore, when rats are supplemented with additional ω-3 FAs in their diet, they display significantly decreased immobility compared to rats fed a normal diet (Ferraz et al., 2008; Huang et al., 2008) indicating ω-3 FA supplementation may assist in alleviating depressive behavior in rodents. The literature is mixed when describing the associations of ω-3 FAs on depression in human subjects. Data from a health survey of Norwegian adults revealed that those who consume cod liver oil which is high in ω-3 FAs were less likely to experience depression (Raeder et al., 2007). Similar results were found in pregnant English women, where lower ω-3 FA intake was associated with increased depressive symptoms (Golding et al., 2009). However, a recent review of 26 studies reports that ω-3 FAs only promote a small to moderate difference in depressive symptoms (Appleton et al., 2007). Other researchers have discovered more specific findings when examining this relationship. For instance, ω-3 FAs may be beneficial to depressive symptoms for males but not females (Murakami et al., 2010). Likewise, meta-analyses of experimental studies are also mixed. One meta-analysis examining 13 randomized placebo-controlled trials found no relationship between depression and ω-3 FAs (Bloch & Hannestad, 2012) while another meta-analysis reviewing 13 randomized placebo-controlled trials describe a significant meta-analytic effect (Mocking et al., 2016). A more
nuanced meta-analysis investigating randomized controlled trials (RCT) of ω-3 FAs as treatment, separated patients with a major depressive disorder (MDD) \((n = 11)\) from patients with depressive symptoms without a MDD diagnosis \((n = 8)\), and found that ω-3 FAs demonstrated clinical benefits as compared to placebo in both MDD groups (Grosso et al. 2014b).

**ω-3 FAs and Anxiety**

The effects of ω-3 FAs on anxiety have been less studied than depression and the research is limited to mainly correlational studies, with only a handful of intervention studies in human subjects. In the animal literature, ω-3 supplementation has been shown to decrease anxiety in non-humans. For instance, a DHA enriched diet has shown to reduce anxiety in mice as measured by the light-dark transition test (Carriè et al., 2002), while a diet enriched with EPA has shown to decrease anxious behavior (e.g., less time spent in arms of elevated maze) in rats (Song, Li, Leonard, & Horrobin, 2003). The impact of ω-3 FAs has also been examined among pregnant rats. Pregnant rats assigned to a ω-3 enriched diet displayed decreased anxiety-like behavior (e.g., increased time spent in the open arms of the elevated plus maze test) (Chen & Su, 2012). Another study by Chen & Su (2013) tested whether a diet deficient in ω-3 at either a pre-weaning stage (embryo-weaning at 3 weeks) as compared to a post-weaning stage (3-10 weeks) would lead to anxiety like behaviors in male offspring. It was found that an ω-3 deficient diet during the pre-weaning period increased anxiety like behavior in the offspring later in life, as compared to the offspring fed the ω-3 deficient diet during the post-weaning stage. These results suggest the importance of ω-3 consumption during pregnancy to ensure healthy development and to ameliorate anxiety.

Although limited, within human subjects, findings of observational and experimental studies examining ω-3 FAs on anxiety indicate encouraging effects. Similar to animal studies, ω-3
3 FAs have been shown to have therapeutic benefits for anxiety symptoms in human samples (Ross, 2009). Several studies have examined the relationship between ω-3 FAs and anxiety in women. In a community-based sample of Australian women, Jacka and colleagues (2013) discovered that women with high levels of DHA intake had a 50% reduced likelihood of a current anxiety disorder (e.g., panic disorder, agoraphobia, social phobia, specific phobia, generalized anxiety disorder). Indeed, pregnant women with lower levels of DHA may have a stronger likelihood of having a current anxiety disorder compared to pregnant women with higher levels of DHA (Verly-Miguel et al., 2015). In addition, pregnant women who consumed diets that included ω-3 FAs were more likely to have lower levels of self-reported anxiety symptoms as compared to pregnant women who did not consume any ω-3 FAs in their diet (dos Santos Vaz et al., 2013). Further, having an anxiety disorder diagnosis, and the severity of the anxiety disorder were associated with the lowest amount of EPA and DHA levels (Liu et al., 2013). One study investigated the effects of ω-3 FAs on eleven patients diagnosed with obsessive-compulsive disorder (OCD). Patients were randomly assigned to receive six weeks of a placebo and six weeks of EPA 2 grams, in counterbalanced order (Fux, Benjamin, & Nemets, 2004). Results demonstrated that as compared to the placebo, supplementing with EPA did not produce any differences in anxiety. One explanation for the lack of differences between the supplements may be the fact that all participants were stabilized on a selective serotonin reuptake inhibitor. Another study found that there were lower concentrations of ω-3 FAs in patients with social anxiety disorder and that this was also associated with greater symptom severity compared to a control group (Green, Hermesh, Monselise, Marom, Presburger, & Weizman, 2006). While few experimental studies have been conducted, the research examining the impact of ω-3 FAs on anxiety indicates promising results.
Potential Pathways Mediating the Effect of ω-3 FAs on Depression and Anxiety

What is Inflammation?

Inflammation is a biological response to bodily injury or infection and is part of the innate immune response (Calder, 2006). The inflammatory response allows immune cells such as granulocytes, monocytes, and macrophages to destroy invading pathogens and remove cellular debris to prepare for future repair. In addition, the inflammatory response involves a release of cytokines, which are proteins produced by monocytes and macrophages to regulate immune and inflammatory reactions (Zhang & An, 2009). However, a prolonged or uncontrollable inflammatory response can result in tissue damage and lead to inflammatory diseases (Calder, 2006).

Importance of Inflammation on Depression and Anxiety

Mental health and inflammation have shown to be associated with one another, and it has been theorized that the release of cytokines as a part of inflammatory response serves a critical role in depression (Maes et al., 2009). Depression has been linked to increased production of proinflammatory cytokines (e.g., TNF-α, IL-6). Two meta-analyses of cross-sectional studies (Dowlati et al., 2010; Howren, Lamkin, Suls, 2009) suggest that inflammatory markers, specifically cytokines TNF-α, IL-1, and IL-6, are elevated in depressed patients. Furthermore, a meta-analysis of longitudinal studies shows a small but significant effect of C-reactive protein (CRP) on depression (Valkanova et al., 2013). One recent study found that subjects with MDD not on medication has significantly higher levels of IL-6 and TNF-α compared to control subjects (Lindqvist et al., 2017).

The relationship between inflammation and anxiety has been less studied. However, one study of older adults used autoregressive cross-lagged panel models to show that levels of CRP
in dried blood spots significantly predicted not only depression, but also anxiety and stress five years later (Das, 2016). Within an adolescent sample it has been found that there is a linear, dose-dependent relationship between levels of CRP and percent of adolescents with GAD (Khandaker, et al. 2016). Furthermore, the adolescents in the top third of serum CRP levels, were five times more likely to develop GAD than those in the bottom third. Adolescents with internalizing disorders (e.g., MDD, generalized anxiety disorder (GAD), separation anxiety disorder, social anxiety disorder or panic disorder) demonstrate significantly higher IL-6 as compared to adolescents without internalizing disorders (da Silva et al., 2017). As evidence indicates that the inflammatory process may elicit anxiety and depressive symptoms and disorders, it is important to explore novel approaches to analyze this relationship.

**Relationship between ω-3 FAs and Inflammation**

It has been established that ω-3 and ω-6 FAs play a role in inflammation as a meta-analysis of 68 randomized control trials (RCTs) discovered that ω-3 supplementation was associated with reduced levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and CRP in healthy participants as well as participants with chronic non-autoimmune diseases and chronic autoimmune diseases (Li, Huang, Zheng, Wu, & Li, 2014). ω-3 and ω-6 FAs affect inflammation by being metabolized into signaling molecules (Calder, 2006) that include prostaglandins, thromboxanes, lipoxins, and leukotrienes (Smith, 1989) as well as protectins, resolvins, and maresins (Serhan, Chiang, Dalli, & Levy, 2015). Increased consumption of ω-3 FAs have been found to suppress the release of proinflammatory eicosanoids and produce anti-inflammatory eicosanoids, leading to reduced inflammation (Wall, Ross, Fitzgerald, & Stanton, 2010).
Understanding how ω-3 FAs biologically interact with immune cells will elucidate how they produce anti-inflammatory effects. Omega-3 FAs play many roles in immune cell functioning such as modifying membrane proteins, modifying membrane composition and fluidity of immune cells, influencing gene expression, and producing lipid mediators (Calder, 2008). Dietary PUFAs undergo a variety of enzymatic reactions before they are converted into eicosanoids, or a family of fatty acids that act as local hormones and influence a variety of biological functions including cell growth and metabolism (Smith, 1989). However, the long-chain fatty acids and their functions depend on the PUFA that they originate from. The EFA from the ω-6 series, LA (18:2n-6) is converted to the twenty-carbon long chain fatty acid AA (20:4n-6) while the EFA from the ω-3 series, ALA (18:3n-3), is converted to the twenty-carbon long chain fatty acid EPA (20:5n-3).

The relationship between ω-3 and ω-6 FAs is not always straightforward. Metabolites of the twenty carbon ω-6 FA AA can exert proinflammatory or pro-resolving actions depending on the specific metabolite and the context (Calder, 2006; Poorani et al., 2015). Prostaglandin E2, for example, contributes not only to the initiation of inflammation (Ricciotti & FitzGerald, 2011) but also to the resolution of inflammation by triggering the production of lipoxin A4, another metabolite of AA (Chan & Moore, 2010; Poorani et al., 2015). Another twenty carbon ω-6 FA, dihomo-γ-linolenic acid (DGLA) inhibits inflammation through its conversion to prostaglandin E1. In contrast, the ω-3 FA EPA inhibits the initiation through its conversion to prostaglandin E3 (Wall, Ross, Fitzgerald, & Stanton, 2010). Moreover, the ω-3 FA DHA promotes the resolution of inflammation through its conversion to resolvins, protectins, and maresins which are vital in the resolution of inflammatory responses (Serhan, 2007). EPA also generates similar metabolites in the presence of aspirin. These pro-resolving compounds assist the return of a tissue to
homeostasis and downregulate proinflammatory cytokines (Bannenberg & Serhan, 2010; Schwab, Chiang, Arita, & Serhan, 2007).

Overall, ω-6 FAs contribute both to the initiation, prevention, and resolution of inflammation, while ω-3 FAs are more straightforwardly anti-inflammatory and pro-resolution. Thus, a balanced ratio of consumption of ω-3 and ω-6 FAs is important. Currently, the western diet has a high ω-6 to ω-3 ratio of approximately 20:1, fostering the development of various diseases such as cardiovascular disease, obesity, and diabetes (Simopolous, 2016). The ideal ratio of ω-6 to ω-3 is 4:1 or 1:1 (Simopoulos, 2008), or somewhere in between. Consumption of fewer ω-6 or improving the intake of ω-3 from food or supplements may improve health by supporting the proper regulation of inflammation.

Although the consumption of a balanced ratio of ω-3 and ω-6 FAs is important, the measurement and reporting of this metric should be interpreted with caution. Harris (2006) reports that a ratio does not allow one to have a clear picture of the absolute value of each of the ω-3 and ω-6 FAs. Furthermore, ω-6 FAs have various physiological properties, which some contributing to the resolution of inflammation and thus should not be compiled together into a ratio with ω-3 FAs (Harris, 2006).

**ω-3 FAs, Inflammation, and Mental Health**

There has been an expansion of empirical research on the mechanisms underlying the relationship between ω-3 FAs and depression (Müller et al., 2015; Su, Wang, & Pae, 2013), with one hypothesized mechanisms of action being the inflammatory response. See Figure 1.

Decreased levels of ω-3 FAs have been found to increase the amount of proinflammatory cytokines and increase depression and anxiety symptoms. For example, university students with low levels of ω-3 FAs under stress displayed increased levels of inflammatory cytokines
compared to controls (Maes, 2000). In addition, adult patients with clinical depression have shown to have lower levels of ω-3 FAs and greater levels of inflammatory markers as compared to control patients (Baek & Park, 2013). Compared to a control group, women with perinatal depression displayed decreased levels of ω-3 FAs and significantly greater levels of TNF-α (Chang et al., 2017).

The impact of ω-3 FAs in relation to inflammatory markers and anxiety symptoms have been less studied. Kiecolt-Glaser and colleagues (2011) examined whether ω-3 FAs would decrease proinflammatory cytokine (IL-6) and anxiety symptoms in a sample of 68 medical students. It was found that the participants who received the ω-3 FA supplements demonstrated a 14% reduction in stimulated IL-6 and a 20% decrease in anxiety scores as compared to a placebo. Although not significant, the authors note that there was a comparable reduction in stimulated TNF-α levels as well.

**What is the Gut Microbiome?**

The human gut microbiome is a collection of trillions of microorganisms (e.g., bacteria, fungi) that reside in the gastrointestinal tract. Each individual has a unique gut microbiome and many factors influence the composition of the gut microbiome including age, sex, and dietary habits. The nutritional value of food is influenced by the structure of an individual’s gut microbiome, and food in turn shapes the microbiome (Kau et al., 2012). The gut microbiome plays a notable role in physical and mental health and alterations within the gut microbiome are associated with the development of various chronic conditions. Thus, it is hypothesized that in addition to the inflammatory response, the gut microbiome may be a potential mechanism underlying the relationship between ω-3 FAs and depression and anxiety.
Importance of the Gut Microbiome for Depression and Anxiety

Additionally, developments have been made in the past decade regarding our understanding of how the brain communicates with the human gastrointestinal tract. Recent research highlights an expanded system: the brain-gut-microbiota axis, which posits that the microbiota in the gut also impacts the central nervous system, which in turn influences neurological functioning, inflammation, and emotion-related behaviors (Wong et al., 2016).

Although novel, there is already extensive research on the brain-gut axis and the bidirectional relationship between the brain and the gastrointestinal tract (GIT) (Grenham et al., 2011; Mayer, 2011). Studies of germ-free gnotobiotic mice (where only known strains of microorganisms are present) show how essential the gut microbiota is not only to the development of a healthy gut, but also to the development of a healthy central nervous system. Animal studies show that when experiencing high levels of stress, animals have altered microbiome profiles (Bailey et al., 2011; Cryan & Dinan, 2012). Furthermore, clinical studies have shown that the administration of the probiotic, Lactobacillus rhamnosus, is associated with reduced anxiety- and depressive-like behavior in rats (Bravo et al., 2011).

These studies have been expanded to human subjects and the gut microbiome appears to play a large role in human cognitive and emotional functioning (Dinan, Stilling, Stanton, & Cryan, 2015). Adults administered probiotics (Lactobacillus helveticus and Bifidobacterium longum) demonstrated improved psychological distress symptoms over a 30-day period (Messaoudi et al., 2011). In another human study, infants supplemented with probiotics had no diagnosis of Asperger’s Syndrome and/or attention deficit hyperactivity disorder at the age of 13, while 17% of children who received placebo formula developed a diagnosis of a developmental disorder (Pärty et al., 2015). Growing evidence suggests that the gut microbiome promotes or
prevents inflammatory responses which in turn may lead to neuropsychiatric disorders (Robertson et al., 2016).

**Effect of ω-3 FAs on the Gut-Microbiome**

Dietary habits have been found to largely influence the structure of the microbiome (David et al., 2014; Kau, 2012). Novel evidence has shown that ω-3 FAs have a significant impact on gut microbiota (Yu et al., 2014). One study found that mice supplemented with ω-3 FAs have more anti-inflammatory bacteria (e.g., lactobacillus and Bifidobacterium) compared to mice with deficient ω-3 FA (Robertson et al., 2016) as well as decreased levels of inflammation and improved gut microbiota profiles (Kaliannan et al., 2015; Myles et al., 2014). Another study examining the impact of ω-3 FAs on gut microbiota in early-life stress found that ω-3 FA supplementation restored the damaged gut microbiota of maternally separated female rats (Pusceddu et al., 2015). Collectively, these findings imply that diet may influence inflammation either directly or indirectly through alterations in the gut microbiome, which then may increase risk for depression and anxiety.

Initial studies have examined how ω-3 FAs alter gut microbiota profiles mainly in animal samples (e.g., mice, piglets) (Andersen, Mølbak, Thymann, Michaelsen, & Lauritzen, 2011; Yu et al., 2014) with limited studies examining human samples (Andersen, Mølbak, Michaelsen, & Lauritzen, 2011). Although the relationship between ω-3 FAs and the gut microbiome within human samples is relatively unknown, studies are beginning to explore this association. One study by Kankaanpää and colleagues (2002) found that probiotic supplemented formula led to changes in PUFA composition among infants as compared to infants consuming regular formula. For example, Bifidobacterium supplemented formula increased ALA through increased absorption (Kankaanpää et al., 2002). Studies focus on a variety of bacteria and demonstrate
mixed results; however, evidence reveals that ω-3 FAs have a therapeutic impact on gut bacteria (Li et al., 2011), which may mediate the physical and psychological benefits of ω-3 FA. Further investigation of these relationships is warranted within human samples given the implications on mental health.

ω-3FAs, Gut-Microbiota, Inflammation and Mental Health

Many of the aforementioned studies suggest that ω-3 FAs affect mental health through inflammatory responses or the gut-microbiota. However, the literature is beginning to look at the dynamic interplay between these variables and how diet plays a critical role in the gut microbiome, inflammatory responses, and mental health (Sandhu et al., 2017). One proposed hypothesis by Logan and Katzman (2005) is that probiotics and ω-3 FAs may have a bidirectional relationship, where ω-3 FAs may alter the types of gut bacteria produced and gut bacteria can impact the amount of ω-3 FA levels. Furthermore, researchers have hypothesized that the absence of healthy bacteria may lead to the initiation of the inflammatory response, and consequently leading to depression (Berk et al., 2013). Based on the literature, it is our scientific premise that ω-3 FAs can influence the gut microbiome by increasing healthy gut bacteria and subsequently reducing inflammatory cytokines, leading to decreased anxiety and depressive symptoms. This hypothesis suggests a serial mediation model can be tested, where the predictor exerts an effect on the outcome through two mediators linked in a causal chain (Hayes, 2015). Taken together, these pathways have the potential to explain the ways in which ω-3 FAs are related to anxiety and depressive symptoms.
Quality of Current Research

The present state of the research involving ω-3 FAs and mental health outcomes demonstrate a great amount of heterogeneity. There are at least three important ways that future research can test how ω-3 FAs may impact psychological health.

Stringent Testing of Dosage and Type of ω-3 FAs

Each type of ω-3 FA (i.e., ALA, EPA, and DHA) has a different biochemical make-up and may have different implications on mental health outcomes over different periods of time. Thus, controlling for the specific type of ω-3 FA administered and length of intervention would enhance the literature allowing for more specific conclusions to be made. Moreover, studies contain a wide range of dosages, although, most studies administer supplements higher than a typical (or over the counter) ω-3 supplement which contains approximately 300 mg of EPA and DHA combined (Wall et al., 2010). One meta-analysis concluded that when EPA supplementation was 200-2200 mg more than DHA, beneficial effects were found in depressive symptoms (Sublette, Ellis, Geant, & Mann, 2011). Currently, there are no established guidelines reporting the ideal balance of ω-3 dosages for mental health symptoms.

Assessing Daily Dietary Intake

While administering ω-3 FAs to participants, intervention studies do not typically assess the diet of their subjects. Utilizing food frequency questionnaires or food diaries would provide a way to control for what participants consume in addition to the treatment intervention. This would increase our understanding of the impact of ω-3 FAs on depression and anxiety, as dietary sources contain different amounts of each type of ω-3 FA (Howe, Meyer, Record, & Baghurst, 2005; Wall et al., 2010). The predominant types of fatty acid consumed on a regular basis depend on geographical region (Food and Agriculture Organization of the United Nations, 2010).
For instance, dietary intake of ω-3 FAs will vary based on regional location such as a coastal or non-coastal region (Al-Numair, Lewis, & Evans, 2005) which can influence research findings.

**Consistent Measurement of Psychological Symptoms or Disorders**

Reviews conducted by Su et al. (2015) and Grosso et al. (2014a) elucidate that meta-analyses done within this area of research are sensitive to heterogeneity among populations, measures, and interventions may not conclude accurate results. However, when the literature systematically reviews a homogenous sample, such as patients diagnosed with MDD, results strongly show the efficacy of ω-3 FAs on depression. In addition, when looking at studies examining ω-3 FAs and anxiety, there are diverse measures of anxiety utilized ranging from general anxiety symptoms to more specific anxiety (e.g., test anxiety). It is important to accurately assess the type of psychological symptoms since different symptoms may modify the effectiveness of ω-3 FAs, and thus would determine the specific patient populations that ω-3 FAs would be recommended for.

**Current Project Aims and Hypotheses**

Although there are many advances in modern medicine, the rate of psychiatric illness continues to rise. Expanding research efforts to incorporate additional factors that may improve psychological health, such as diet, can potentially enhance the development, management and prevention of anxiety and depression. Many studies have examined the relationship between ω-3 FAs and depression and anxiety demonstrating a positive impact on psychological symptoms. Yet few studies have examined the potential mechanisms underlying the relationship between ω-3 FAs and depression and anxiety.

The present study aims to explore the potential mechanisms underlying the relationship between ω-3 FAs, and stress, anxiety and depression symptoms, with the hypothesized
mechanisms being inflammation and the gut microbiome. Many of the variables outlined have been examined separately in animal and human samples, however, no one study has examined the associations between ω-3 intake, gut microbiota functioning, inflammatory markers, and stress, anxiety and depression outcomes in a human sample. Given that research has established that diet has a considerable impact on both physical and mental health, the present study aims to expand the literature by understanding the ways in which these relationships occur. Additionally, since this is the first study examining these relationships, paired with evidence that males and females exhibit differences in ω-3 levels (Kitson, Stroud, & Stark, 2010) and presentation of gut bacteria (Jašarević, Morrison, & Bale, 2016) due to differences in sex hormones; we will recruit an all female sample. Elucidating these relationships will shed light onto how dietary intake can influence our biological functioning and subsequently, mental health conditions. This understanding will, in turn, be able to shape the way we understand how psychological conditions develop and can help influence the prevention and treatment of anxiety and depression.

The specific aims for the proposed study are two-fold: 1) To examine the cross-sectional relationship between ω-3 FAs, gut microbiome bacteria and inflammatory markers, and stress, anxiety and depressive symptoms. It is hypothesized that lower ω-3 intake will be associated with greater stress, anxiety, and depression symptoms. Lower ω-3 intake will be associated with deceased levels of Lactobacillus and Bifidobacterium and increased inflammation (TNF-α, IL-6, IL-8, IL-1β). Decreased levels of Lactobacillus and Bifidobacterium and increased inflammation will be associated with greater stress, anxiety, and depression symptoms. 2) To examine if the gut microbiome and inflammatory markers mediate the relationship between ω-3 intake and stress, anxiety and depressive symptoms.
It is hypothesized that ω-3 intake will have an indirect association with anxiety and depressive symptoms through the gut microbiome (Lactobacillus and Bifidobacterium) and inflammatory markers (TNF-α, IL-6, IL-8, IL-1β) (see Figure 5).
CHAPTER II: METHODOLOGY

Participant Characteristics

Healthy adult females ages 18 and older and fluent in English were recruited to participate in the present study ($N = 79$). Participants were students, faculty, and staff from Brooklyn College or from the local community. Participants were excluded if they were pregnant, have been diagnosed with an inflammatory or endocrine illness, engaged in heavy alcohol use (more than two glasses of alcohol per day) or heavy smoking (more than eight cigarettes per day), or have been in psychological or psychiatric treatment for less than 6 months.

Participant demographic information is presented in Table 1. Participants ages ranged 18 to 42 years old ($M = 21.7$, $SD = 4.1$), the sample was all female, and they identified their ethnicity as primarily Black (25.3%), White (24.1%), and Hispanic/Latino (24.1%). The majority of participants reported single status (89.9%) and one third were part of a household income less than $25,000 (34.2%). More than half of the participants (68.4%) were born via vaginal birth as compared to cesarean section (25.3%). Approximately one third of participants (32.9%) reported they were fed both breast milk and formula as a baby, one fourth of participants (24.1%) reported being breast-fed, and formula-fed (24.1%), and approximately one fifth of participants (17.7%) were not sure. The majority of participants are not currently taking an oral contraceptive (87.3%) and denied past use of an oral contraceptive (75.9%).

Descriptive statistics of self-report measures are presented in Table 2. Based on the established clinical cutoff scores, 39.2% of participants ($n = 31$) reported no to minimal depressive symptoms, 41.8% of participants ($n = 33$) reported mild depressive symptoms, 15.2% of participants ($n = 12$) reported moderate depressive symptoms and 3.8% of participants ($n = 3$) reported severe depressive symptoms.
Individuals with past oral contraceptive use reported higher stress symptoms on the PSS ($M = 23.11, SD = 4.81, n = 19$) compared to those who have not used oral contraceptives ($M = 19.05, SD = 6.87, n = 60$) ($t = 2.86, df = 43.15, p = 0.006$). No other significant differences were noted on self-report measures as a function of ethnicity, marital status, income, method of birth (e.g., vaginal or cesarean section), breast fed or formula fed, or current oral contraceptive use.

Participant’s average ω-3 index, DHA, EPA, and ALA fatty acid percentage values are presented in Table 3. No significant differences were noted on the ω-3 index as a function of marital status, income, method of birth, breast fed or formula fed, or current or past oral contraceptive use. There was a significant effect of ethnicity on DHA percentage values ($F(5,73) = 6.82, p < 0.001$) and ω-3 Index levels ($F(5,73) = 6.02, p < 0.001$). Post hoc-comparisons using a Tukey HSD test showed that DHA values for Asian/Pacific Islander participants ($M = 3.49, SD = 0.66$) was significantly higher compared to White ($M = 2.23, SD = 0.56$), Hispanic ($M = 2.53, SD = 0.69$), or Mixed ethnicity ($M = 2.24, SD = 0.53$) participants. DHA levels were also significantly higher in Black participants ($M = 2.90, SD = 0.67$) compared to White participants. Post-hoc comparisons revealed similar findings for ω-3 Index levels where the mean was significantly higher for Asian/Pacific Islander participants ($M = 5.97, SD = 1.09$) compared to White ($M = 4.32, SD = 0.66$), Hispanic ($M = 4.70, SD = 0.91$), or Mixed ethnicity ($M = 4.46, SD = 0.80$) participants. Omega-3 Index levels were also significantly higher in Black participants ($M = 5.22, SD = 0.94$) compared to White participants ($M = 4.32, SD = 0.66$). These differences in ethnicity were unsurprising as the literature has demonstrated that individuals of Asian descent exhibit higher levels of ω-3s than White individuals (Xiao et al., 2016). Similarly, a prospective cohort study in the United States has shown that Black participants had higher ω-3
FAs (Villegas, Takata, Murff, & Blot, 2016) than White participants or those identifying as another ethnicity.

The mean concentration of each inflammatory marker (IL-1β, IL-6, IL-8, TNF-α) in pictograms per milliliter (pg/mL) are presented in Table 3. No significant differences in inflammatory markers were noted as a function of marital status, ethnicity, income, method of birth, breast fed or formula fed, and current or past oral contraceptive use.

The average percentages of abundance of gut bacteria (Lactobacillus and Bifidobacterium) are presented in Table 3. No significant differences were noted on gut bacteria between ethnicity, marital status, income, method of birth, breast fed or formula fed, or current or past contraceptive use. A significant effect of income level was noted on Lactobacillus ($F(6,60) = 2.50, p < 0.03$). Post hoc-comparisons using Tukey HSD indicated that the participants with a household income of $75,000-$99,999 ($M = 5.42, SD = 9.09$) has significantly higher abundance of Lactobacillus compared to participants with a household income of less than $25,000 ($M = 0.68, SD = 1.46$), $35,000-$49,999 ($M = 0.04, SD = 0.08$), and $50,000-$74,999 ($M = 0.37, SD = 0.52$). It is important to note that this difference in income level could be a type I error given that the household income group of $75,000-$99,000 only has three participants.

**Recruitment**

Participants were recruited via flyer postings around the Brooklyn College campus. Study information was disseminated via electronic mail to reach Brooklyn College students and faculty members. We also used snowball sampling, where existing participants recruited future participants, and online advertisement posting websites (e.g., Craig’s list). All participants who express interest in participating in the study were briefly screened to determine study eligibility.
Study Procedures

Once screened as eligible, participants were scheduled to come to Brooklyn College for their assessment. Participants were emailed 24 hours prior to remind them of their appointment and of the saliva collection instructions (e.g., do not drink alcohol or smoke 12 hours prior to the appointment, and do not eat, drink caffecinated beverages, brush their teeth or use mouthwash one hour prior to the appointment). After arriving to the lab, participants completed the informed consent procedure with a research assistant. Next participants were asked about saliva-collection-specific criteria. If the participant has complied, a saliva sample was taken to measure inflammatory cytokines. Participants provided a minimum of 0.5ml and up to 2ml of a saliva sample. Saliva samples were collected using passive drool, which means that participants were asked to drool into a small tube, and used a straw to facilitate saliva collection. Once collected, saliva samples were immediately placed into a freezer approximately -20 degrees Celsius until they were ready to be analyzed.

Next, the dried blood spot sample was collected. Each participant had their sample collected by a trained research assistant and materials used were from an individual sealed kit. Participants were asked to wash their hands with warm water and hold them at their sides for 20 seconds to increase circulation to their fingers. A research assistant cleaned the participant’s middle or ring finger with an alcohol swab and then used a sterile lancet positioned on the side of the finger and pressed firmly until a click was heard and the needle was inserted. Blood was collected on the finger and the finger was lightly squeezed to facilitate blood flow. The participant's’ finger was pressed on the center of the sample collection card. This was repeated until the entire collection circle was filled with blood. Afterwards pressure was applied to the finger with a gauze pad and a bandage was applied. Once the blood spot had dried for 15-20
minutes the collection card immediately placed into a freezer approximately -20 degrees Celsius until they were ready to be analyzed.

Following the saliva and blood sample collection, participants completed the self-report measures (e.g., demographic history, PSS, STAI, BDI) on a laptop in the research lab. After all self-report measures were completed, the research assistant gave the participant a uBiome stool sample kit with a unique ID number. Sampling was done privately in a bathroom on campus or in their home. Collection of stool sample takes less than two minutes and is a noninvasive procedure. Participants were instructed to swab their toilet paper, put the swab in a tube provided, and shake the tube for one minute. Then, participants placed their sample in a pre-paid return mailer. Once complete, the sample can be stored at room temperature for up to one month, however, the participant was instructed to mail out the sample to uBiome by placing in a mailbox promptly after collection. Any personal health information that participants provided are protected by the uBiome privacy practices; uBiome is required by the Health Insurance Portability and Accountability Act of 1996 (HIPAA) to keep all health information confidential. Once instructions were discussed and understood participants were given $25 for their participation signed a receipt acknowledging their payment.

**Self-Report Measures**

Demographic information collected included: age, gender, race, education level, past and present geographic location, food allergies, dietary restrictions, specific amount of smoking and alcohol use, whether the participant was born via cesarean section or via vaginal delivery, whether the participant was breast fed or formula fed, and past or current treatment for anxiety.

**Perceived Stress Scale (PSS):** The PSS is a widely used instrument to assess perceived stress over the past month (Cohen, Kamarck, & Mermelstein, 1994). The PSS is a 10-item
measure assessing the degree in which particular areas in one’s life are perceived as stressful. Items are rated on a 5-point Likert scale (0 = never, to 4 = very often). Total PSS scores are calculated by reverse-coding four reverse-worded items and then summing across all scale items. Total scores can range from 0-40, with higher scores reflecting higher perceived levels of stress. The PSS demonstrates good reliability and validity (Siqueira Reis, Ferreira Hino, & Romélio Rodriguez-Añez, 2010). The measure had good internal consistency in the present sample ($\alpha = 0.87$) on the PSS.

*State-Trait Anxiety Inventory (STAI)*: Anxiety was measured using the STAI (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). The STAI is a self-report measure that assesses state and trait anxiety in patients. The inventory has 40 questions, 20 assessing state anxiety or in a specific situation (STAI-S) and 20 assessing general trait anxiety or a stable disposition (STAI-T). Items are rated on a four-point scale (1 = almost never, to 4 = almost always) with higher scores indicating greater anxiety. The STAI has shown to be highly reliable and valid (Vitasari, Wahab, Herawan, Othman, & Sinnadurai, 2011). The measure demonstrated excellent reliability ($\alpha = 0.95$) in the present sample.

*Beck Depression Inventory (BDI)*: To assess depressive symptoms, the BDI was administered (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961). The BDI is a 21-item self-report measure that assesses clinical severity of depressive symptoms. There are four possible responses for each item ranging from 0-3, depending on intensity. Scores are calculated by summing across the 21 items. Total depression scores are categorized as minimal (0–9), mild (10–18), moderate (19–29) and severe depression (30–63). The BDI has been found to be a reliable and valid measure (Reynolds & Gould, 1981). The BDI had high reliability ($\alpha = 0.87$) in the present sample.
**Omega-3 Index & Omega-3**

Omega-3 FAs was measured via dried blood spot samples from a finger prick using the Omega-3 Index Kit provided by and analyzed by Omega Quant Laboratories (OmegaQuant Analytics, Sioux Falls, SD). An Omega-3 Index will be calculated which reflects the EPA and DHA content of the red blood cells. This will be reported as a percentage of the total identified fatty acids in the blood. In addition, a full blood fatty acid profile will be provided with 24 individual fatty acid values. This method has been previously used in assessing Omega-3 Index in patients with depression (Baghai et al., 2011), U.S. servicemen (Johnston, Deuster, Harris, MacRae, & Dretsch, 2013) and vegans (Sarter, Kelsey, Schwartz, & Harris, 2015).

**Inflammatory Measurement**

Proinflammatory biomarkers were assessed via salvia sample. Specific biomarkers that were measured include TNF-α, IL-6, IL-8, IL-1β. Salvia was collected using passive drool and analyzed by Salimetrics Laboratory using enzyme-linked immunosorbent assay (ELISA) (Salimetrics, LLC, State College, Pennsylvania). In order to collect the most accurate assessment of inflammatory markers, participants were instructed not to drink alcohol or smoke 12 hours prior to testing. In addition, they were instructed not to eat, drink caffeinated beverages, brush their teeth or use mouthwash one hour prior to testing. The results from each biomarker included two reports from each participant and the average in pictograms per milliliter (pg/mL).

**Gut Microbiome**

Levels of gut bacteria, Lactobacillus and Bifidobacterium, were measured from participants stool samples. Participants were given a uBiome stool sample kit and provided instructions as to how to submit their stool sample. Participants used a sterile cotton swab to transfer fecal matter into a vial containing a lysis and stabilization buffer that protects DNA
during transportation. Each kit has a unique Kit ID associated with it and was assigned to a participant. Therefore, the participant did not include any identifying information when mailing out their fecal sample. Data from the stool sample was collected by uBiome and linked to a research account created by the PI. Lactobacillus and Bifidobacterium levels of each participant were displayed as a percentage of abundance compared to the general population to determine if they are above or below average.

**Statistical Analysis**

Power analyses were conducted using G Power software (Faul, Erdfelder, Buchner, & Lang, 2009). Statistical analysis was conducted in IBM SPSS Statistics Version 25. The goal was to have 60 participants complete the current study. We estimated a 20% non-compliance rate (e.g., not providing a stool sample), thus, we aimed to recruit a projected estimate of 80 participants to achieve our goal. A sensitivity power analysis was calculated based on a desired power of .80 and the predetermined sample size (Faul, Erdfelder, Lang, & Buchner, 2007). For a linear multiple regression with three predictors, based on 60 participants, $\alpha = 0.05$, and a power of .80, a medium effect size ($f^2 = 0.19$) will be able to be detected. Effect sizes can be classified as small ($f^2 = 0.02$), medium ($f^2 = 0.15$), or large ($f^2 = 0.35$).

**Aim 1:** To investigate the cross-sectional associations between $\omega$-3 FAs (EPA/DHA), stress, anxiety and depressive symptoms (PSS, STAI, BDI), inflammatory markers (TNF-$\alpha$, IL-6, IL-10) and gut bacteria (Lactobacillus and Bifidobacterium), Pearson correlation analyses will be conducted. A Bonferroni correction will be applied, diving alpha by the number of comparisons being made, which is used when multiple comparisons occur simultaneously in the one analysis to prevent Type I error. Pearson correlation coefficients can be classified as positive or negative, and can range from a very weak relationship ($r = 0.00-0.19$), to a weak relationship ($r = 0.20-$
0.39), to a moderate relationship ($r = 0.40-0.69$), to a strong relationship ($r = 0.70-0.89$), to a very strong relationship ($r = 0.90-1.00$) (Cohen & Holiday, 1982). It is hypothesized there will be a negative association between $\omega$-3 intake and stress, anxiety and depressive symptoms (Aim 1A) given that previous research has found support for this relationship in both animal and human subjects (Carrié et al., 2002; Ferraz et al., 2008; Jacka et al. 2013; Raeder et al., 2007). It is predicted there will also be a negative association between $\omega$-3 intake and inflammation (Aim 1B). Studies have found decreased levels proinflammatory markers correlated to $\omega$-3FA supplementation (Wall, Ross, Fitzgerald, & Stanton, 2010). Furthermore, it is hypothesized there will be a positive association between $\omega$-3 intake and levels of Lactobacillus and Bifidobacterium (Aim 1C) as studies have shown that $\omega$-3 supplementation is related to increased levels of these bacteria mice (Robertson et al., 2016) and supplementation of Bifidobacterium was correlated to increased ALA concentration in human infants (Robertson et al., 2016). We predict there will be a negative association between Lactobacillus and Bifidobacterium and stress, anxiety and depressive symptoms (Aim 1D) since previous studies have shown that supplementation of Lactobacillus was associated with decreased anxious and depressive behavior in rats (Bravo et al., 2011) and decreased distress symptoms in humans (Messaoudi et al., 2011). Lastly, inflammation is hypothesized to have a positive relationship with stress, anxiety and depression symptoms (Aim 1E) as studies have demonstrated inflammatory markers to be positively associated with anxiety and depressive disorders (Khandaker, et al. 2016; Lindqvist et al., 2017).

**Aim 2**: To examine if the gut microbiome and inflammatory markers mediate the relationship between $\omega$-3 intake and stress, anxiety and depressive symptoms, we will utilize the computational and modeling program PROCESS v.2.15 (Hayes 2013) to test both direct and
indirect effects. PROCESS Model 6 was selected to test for serial mediation, where the predictor is modeled to exert an effect on the outcome through two mediators linked in a causal chain (Hayes, 2015). The bootstrapping resampling method was utilized, using 5000 bootstrap samples, this nonparametric method estimates direct and indirect effects and 95% bias-corrected confidence intervals and does not assume normality.

This study aims to investigate the direct and indirect effects of ω-3 FAs on the three dependent variables of stress, anxiety, and depressive symptoms and the mediating role of gut bacteria and inflammation. For the present study, stress, anxiety, and depressive scores were entered as the outcome variable, measures of ω-3 FAs as the predictor variables, and gut bacteria and inflammatory markers as the mediators. To test all combinations of serial mediation, each possible combination will be independently run. With four outcome variables (stress, trait-anxiety, state-anxiety, depression), four predictor variables (ω-3 Index, DHA, EPA, ALA), and six mediator variables (Lactobacillus, Bifidobacterium, and TNF-α, IL-6, IL-8, IL-1b) there are 128 possible mediation analyses (32 separate analyses for each outcome variable).

The first set of analyses will examine the direct and indirect effects of ω-3 FAs, the predictor variable, on stress symptoms, through the two mediators, gut bacteria and inflammation (Aim 2A) (Figure 2). The second set of analyses will examine the direct and indirect effects of ω-3 FAs on anxiety symptoms, through the mediator’s gut bacteria and inflammation (Aim 2B) (Figure 3). Lastly, the third set of analyses will examine the direct and indirect effects of ω-3 FAs on depressive symptoms, through the mediator’s gut bacteria and inflammation (Aim 2C) (Figure 4).

Specifically the following relationships will be analyzed in mediation analysis: 1) the direct relationship between ω-3 and stress, anxiety, and depression symptoms, 2) the direct
relationship between $\omega$-3 and gut bacteria, 3) the direct relationship between $\omega$-3 and inflammation, 4) the direct relationship between gut bacteria and inflammation, 5) the direct relationship between gut bacteria and stress, anxiety, and depression symptoms, 6) the direct relationship between inflammation and stress, anxiety, and depression symptoms, 7) the indirect relationship between $\omega$-3 and stress, anxiety, and depression symptoms through gut bacteria, 8) the indirect relationship between $\omega$-3 and stress, anxiety, and depression symptoms through inflammation, and 9) the indirect effect of $\omega$-3 and stress, anxiety, and depression symptoms through both gut bacteria and inflammation. We will test whether any demographic variables (e.g., race, marital status, income) are significantly related to any outcome variables and if so, they will be entered into the model as covariates given that these relationships have not been previously examined and these affect dietary intake (Al-Numair, Lewis & Evans, 2005; Darmon & Drewnowski, 2008; Franco et al., 2009).
CHAPTER III: RESULTS

Normality

The distributions of study variables were inspected for normality using visual inspection and normality tests of skewness, kurtosis, and Shapiro-Wilk values (Appendix X). Normality was defined as a skew and kurtosis z-score between -2 and 2 (Bulmer, 1979). Report of depression symptoms, state-anxiety symptoms, trait-anxiety symptoms, inflammation data, ω-3 data, and gut bacteria data were all positively skewed. Both square root and logarithmic transformations were conducted (Appendix Y), as these two transformation methods are recommended for positively skewed data (Howell, 2007; Tabachnick, & Fidell, 2007). For the next section (Aim 1), the nonparametric Spearman rank correlation coefficient was used with non-transformed data to examine associations among variable because the data did not meet normality assumptions. Once log transformations were conducted, Pearson correlations were utilized to examine study variable relationships. The results are presented for both analyses for Aim 1.

Aim 1 (5 hypotheses): Bivariate relationships between ω-3 FAs, gut microbiome bacteria, inflammatory markers, and psychological self-report measures.

For the first hypothesis, the association between ω-3 intake and psychological self-report measures (Aim 1A) was examined. There was a negative association between ω-3 index and trait-anxiety symptoms ($\rho (77) = -0.29, p = 0.01$) as well as DHA levels and trait-anxiety symptoms ($\rho (77) = -0.30, p = 0.01$). Contrary to our hypotheses, there was no significant relationships between ω-3 intake and any other psychological measure (see Table 4).

As anticipated, there was a significant negative relationship between ω-3 index and inflammatory marker IL-1β ($\rho (77) = -0.26, p = 0.02$) and between DHA and IL-1β ($\rho (77) = -$
0.24, \( p = 0.03 \)). However, there was no significant relationship between \( \omega-3 \) intake and other inflammatory markers (Aim 1B) (see Table 5). Furthermore, gut bacteria levels were not significantly associated with \( \omega-3 \) intake (Aim 1C) (see Table 6) or psychological self-report measures (Aim 1D) (see Table 7). We found a weak positive association between depression symptoms and the inflammatory marker IL-1\( \beta \) (\( \rho (77) = 0.26, p = 0.02 \)) (Aim 1E) (see Table 8). No other significant relationships were noted between inflammatory markers and psychological self-report measures.

**Log-transformed Correlational Results**

In addition to running Spearman’s correlation, the original data were log transformed to allow for the use of Pearson’s correlation to examine study variable relationships. Results using Pearson correlations on transformed data were similar to findings for Aim 1A using Spearman correlations; there was a negative relationship between trait-anxiety symptoms and both \( \omega-3 \) index \( (r (77) = -0.28, p = 0.01) \) and DHA levels \( (r (77) = -0.30, p = 0.01) \). There was one additional finding for Aim 1A, there was a negative association between depression symptoms and \( \omega-3 \) index \( (r (77) = -0.24, p = 0.03) \) and DHA levels \( (r (77) = -0.24, p = 0.03) \). For the remainder of the hypothesized associations, all results were non-significant. Overall, Pearson correlation results demonstrate a similar negative relationship between trait-anxiety and both \( \omega-3 \) index and DHA. One new association was found between \( \omega-3 \) index and depressive symptoms. There were three associations that were found in the Spearman’s correlation analyses were not found when conducting the Pearson’s correlations in that IL-1\( \beta \) was not associated with \( \omega-3 \) index, DHA or depressive symptoms.
Post-hoc Analysis

As a post-hoc analysis, we examined the relationship between gut bacteria and inflammation which are the proposed mediating variables. No significant relationships were noted between gut bacteria and inflammatory markers (Table 9) using Spearman rank correlation or the Pearson correlation after log-transformation.

Aim 2: Serial mediation to test if gut bacteria and inflammatory markers mediate the relationship between ω-3 intake and psychological self-report measures

Serial mediation models assessed whether gut bacteria and inflammatory markers mediated the relationship between ω-3 intake levels and psychological symptoms. Specifically, there are four outcome variables for psychological symptoms (stress, trait-anxiety, state-anxiety, depression), four predictor variables of ω-3 intake (ω-3 Index, DHA, EPA, ALA), and six mediator variables of gut bacteria and inflammatory markers (Lactobacillus, Bifidobacterium, and TNF-α, IL-6, IL-8, IL-1β). Each possible combination was independently conducted, resulting in 32 analyses for each outcome variable. Mediation results are separated by outcome variables, first stress symptoms, next anxiety symptoms including trait- and state-anxiety, and lastly depression symptoms. Across all 128 serial mediation analyses conducted, no mediation was noted. However, several significant direct effects were detected (see Figure 5 for conceptual model and key for direct effects). The findings for each predictor and outcome will be listed below, regression coefficients reported are unstandardized.

Serial mediation analyses for stress symptoms (Aim 2A).

Omega-3 Index (predictor) and PSS. Across the eight models assessing the effect of the ω-3 index on stress symptoms, three path coefficients were statistically significant (see Table
IL-1β was positively associated with stress symptoms in the model with Lactobacillus ($b = 0.01$, $t(63) = 2.33$, $p = 0.02$) and in the model with Bifidobacterium ($b = 0.01$, $t(63) = 2.46$, $p = 0.02$). In addition, greater abundance of Lactobacillus significantly predicted higher levels of inflammatory marker IL-8 ($b = 57.96$, $t(64) = 2.50$, $p = 0.02$).

**DHA (predictor) and PSS.** Similar results were found in the models assessing DHA on stress symptoms (See Table 11). Higher levels of IL-1β predicted increased stress symptoms when Lactobacillus was a mediator ($b = 0.01$, $t(63) = 2.29$, $p = 0.03$) and when Bifidobacterium was a mediator ($b = 0.01$, $t(63) = 2.44$, $p = 0.02$). Furthermore, Lactobacillus was positively associated with inflammatory marker IL-8 ($b = 59.15$, $t(64) = 2.56$, $p = 0.01$).

**EPA (predictor) and PSS.** With EPA as the predictor, seven path coefficients were statistically significant (see Table 12). Inflammation was positively associated with stress symptoms in four models. IL-1β significantly predicted stress symptoms when Lactobacillus was a mediator ($b = 0.01$, $t(63) = 2.49$, $p = 0.02$) and when Bifidobacterium was a mediator ($b = 0.01$, $t(63) = 2.61$, $p = 0.01$). Additionally, inflammatory marker IL-6 significantly predicted stress symptoms in the models with Lactobacillus ($b = 0.24$, $t(63) = 2.02$, $p = 0.05$) and with Bifidobacterium ($b = 0.24$, $t(63) = 2.06$, $p = 0.04$). Lactobacillus was positively associated with inflammatory marker IL-8 ($b = 57.17$, $t(64) = 2.41$, $p = 0.02$). In two models, higher levels of EPA predicted greater levels of inflammatory marker IL-6 when Lactobacillus was a mediator ($b = 8.27$, $t(64) = 2.12$, $p = 0.04$) and when Bifidobacterium was a mediator ($b = 7.77$, $t(64) = 1.98$, $p = 0.05$).

**ALA (predictor) and PSS.** Serial mediation results with ALA as the predictor were similar to the results examining ω-3 index and DHA as the predictor (see Table 13). IL-1β was positively associated with stress symptoms in the model with Lactobacillus ($b = 0.01$, $t(63) = 2.33$, $p = 0.02$) and in the model with Bifidobacterium ($b = 0.01$, $t(63) = 2.46$, $p = 0.02$). In addition, greater abundance of Lactobacillus significantly predicted higher levels of inflammatory marker IL-8 ($b = 57.96$, $t(64) = 2.50$, $p = 0.02$).
2.59, p = 0.01) and with Bifidobacterium (b = 0.01, t(63) = 2.72, p = 0.01). Furthermore Lactobacillus significantly predicted IL-8 (b = 56.78, t(64) = 2.41, p = 0.02).

**Post-hoc Analysis for PSS.** Given that participants with past oral contraceptive use reported higher stress symptoms compared to participants without past oral contraceptive use, serial mediation models for the PSS were recomputed with past oral contraceptive use as a covariate.

*Omega-3 (predictor) and PSS with Covariate.* Two of the three path coefficients remained significant when adding past oral contraceptive use in the model, IL-1β was positively associated with stress symptoms in the model with Bifidobacterium (b = 0.01, t(62) = 2.11, p = 0.04) and greater abundance of Lactobacillus significantly predicted higher levels of inflammatory marker IL-8 (b = 56.56, t(63) = 2.40, p = 0.02). Inflammatory marker IL-1β was no longer associated with stress symptoms in the model with Lactobacillus. In addition, participants with past oral contraceptive use was associated with higher reported stress symptoms across five of the eight models assessing ω-3 index on stress symptoms.

*DHA (predictor) and PSS with Covariate.* Results were comparable for the models assessing DHA on stress symptoms. Two of the three path coefficients remained significant when adding past oral contraceptive use in the model, IL-1β was positively associated with stress symptoms in the model with Bifidobacterium (b = 0.01, t(62) = 2.09, p = 0.04) and greater abundance of Lactobacillus significantly predicted higher levels of inflammatory marker IL-8 (b = 57.71, t(63) = 2.45, p = 0.02). Similarly, inflammatory marker IL-1β was no longer significantly associated with stress symptoms in the model with gut bacteria Lactobacillus. Past oral contraceptive use positively predicted stress symptoms in four of the eight models analyzed.
**EPA (predictor) and PSS with Covariate.** With EPA as the predictor, all seven path coefficients remained to be statistically significant with past oral contraceptive use was entered as a covariate. IL-1β continued to significantly predict stress symptoms when Lactobacillus was a mediator \((b = 0.01, t(62) = 2.07, p = 0.04)\) and when Bifidobacterium was a mediator \((b = 0.01, t(62) = 2.24, p = 0.03)\). Additionally, inflammatory marker IL-6 continued to significantly predict stress symptoms in the models with Lactobacillus \((b = 0.24, t(62) = 2.15, p = 0.04)\) and with Bifidobacterium \((b = 0.25, t(62) = 2.21, p = 0.03)\). Lactobacillus remained positively associated with inflammatory marker IL-8 \((b = 56.02, t(63) = 2.32, p = 0.02)\). In two models, greater levels of EPA predicted higher levels of IL-6 when Lactobacillus was a mediator \((b = 8.23, t(63) = 2.10, p = 0.04)\) and when Bifidobacterium was a mediator \((b = 7.73, t(63) = 1.96, p = 0.05)\). In addition to past oral contraceptive use being associated with increased stress symptoms across all eight models, it was noted that past oral contraceptive use predicted inflammatory marker IL-1β when Lactobacillus was a mediator \((b = 137.29, t(63) = 1.97, p = 0.05)\).

**ALA (predictor) and PSS with Covariate.** Serial mediation analyses with ALA as the predictor and past oral contraceptive use as a covariate led to the same three significant path coefficients. IL-1β was positively associated with stress symptoms in the model with Lactobacillus \((b = 0.01, t(62) = 2.15, p = 0.04)\) and with Bifidobacterium \((b = 0.01, t(62) = 2.32, p = 0.02)\). Furthermore, Lactobacillus significantly predicted IL-8 \((b = 56.00, t(63) = 2.34, p = 0.02)\). Additionally, past oral contraceptive use being associated with increased stress symptoms across six of the eight models, and past oral contraceptive use predicted inflammatory marker IL-1β when Lactobacillus was a mediator \((b = 149.47, t(63) = 1.83, p = 0.04)\).
Summary for PSS. Four ω-3FA variables (ω-3-index, DHA, EPA, ALA) were tested as predictors of stress symptoms (PSS) in a total of 32 serial mediation models (eight per predictor). Across these models, ω-3FAs did not predict stress symptoms. However, several consistent patterns emerged; higher levels of IL-1β predicted more reported stress symptoms and greater abundance of the gut bacteria Lactobacillus significantly predicted higher levels of the inflammatory marker IL-8. In the models with EPA as the predictor, EPA was positively associated with IL-6 and IL-6 was positively associated with stress symptoms, however there was not a significant indirect effect of IL-6 on the relationship between EPA and stress symptoms. Furthermore, when past oral contraceptive use was entered as a covariate, past oral contraceptive use predicted higher levels of reported stress symptoms across 23 of the 32 serial mediation models. Lastly, there was a positive association between past oral contraceptive use and inflammatory marker IL-1β in two models.

Serial mediation analyses for trait-anxiety symptoms (Aim 2B).

Omega-3 Index (predictor) and STAI-Trait. The direct effect of ω-3 levels on trait-anxiety symptoms, without the influence of the mediators, was significant (see Table 14). The total effect of ω-3 levels on trait-anxiety symptoms was significant ($b = -3.62, t(65) = -2.73, p = 0.01$), which is the sum of the direct and indirect effects of the model. Omega-3 index predicted less trait-anxiety symptoms. One path coefficient was noted to be significant, Lactobacillus was positively associated with inflammatory marker IL-8 ($b = 57.96, t(64) = 2.50, p = 0.02$).

DHA (predictor) and STAI-Trait. With DHA as the predictor, results were similar to ω-3 index results described above. The total effect of DHA on trait-anxiety symptoms was significant ($b = -4.87, t(65) = -2.69, p = 0.01$), as well as a significant direct effect of DHA on trait-anxiety
symptoms across all eight models (see Table 15). In addition, greater abundance of Lactobacillus predicted higher levels of inflammatory marker IL-8 (d) \((b = 59.15, t(64) = 2.56, p = 0.01)\).

**EPA (predictor) and STAI-Trait.** With EPA as the predictor, three path coefficients were statistically significant (see Table 16). In two models, higher levels of EPA predicted higher levels of the inflammatory marker IL-6, when Lactobacillus was entered as a mediator \((b = 8.27, t(64) = 2.12, p = 0.04)\) as well as when Bifidobacterium was entered as a mediator \((b = 7.77, t(64) = 1.98, p = 0.05)\). Additionally, Lactobacillus was positively associated with inflammatory marker IL-8 (d) \((b = 57.17, t(64) = 2.41, p = 0.02)\).

**ALA (predictor) and STAI-Trait.** Greater abundance of Lactobacillus predicted higher levels of inflammatory marker IL-8 \((b = 56.78, t(64) = 2.41, p = 0.02)\) when ALA was the predictor (see Table 17).

**Omega-3/DHA/EPA/ALA (predictor) and STAI-State.** All serial mediation results for the state-anxiety outcome demonstrated the same significant path coefficients as the trait-anxiety results with the exception of the direct and total effects which were not significant. Specifically, greater abundance of Lactobacillus was positively associated with inflammatory marker IL-8 when \(\omega-3\) was the predictor \((b = 57.96, t(64) = 2.50, p = 0.02)\), when DHA was the predictor \((b = 59.15, t(64) = 2.56, p = 0.01)\), when EPA was the predictor \((b = 57.17, t(64) = 2.41, p = 0.02)\) and when ALA was the predictor \((b = 56.78, t(64) = 2.41, p = 0.02)\). Additionally, higher levels of EPA predicted higher levels of the inflammatory marker IL-6, when Lactobacillus was entered as a mediator \((b = 8.27, t(64) = 2.12, p = 0.04)\) as well as when Bifidobacterium was entered as a mediator \((b = 7.77, t(64) = 1.98, p = 0.05)\).

**Summary for STAI-Trait and STAI-State.** Taken together, across the 64 serial mediation models testing \(\omega-3\)FA variables (\(\omega-3\)-index, DHA, EPA, ALA) as predictors of trait- and state-
anxiety symptoms (STAI-Trait and STAI-State) there was a direct and total effect of ω-3 index and DHA predicting less trait-anxiety symptoms but not state-anxiety symptoms. It was consistently found that greater abundance of Lactobacillus predicted higher levels of inflammatory marker IL-8 and higher levels of EPA predicted greater levels of IL-6 across all serial mediation models with trait- and state-anxiety symptoms as the outcome.

**Serial mediation analyses for depression symptoms (Aim 2C).**

**Omega-3 Index (predictor) and BDI.** A significant direct effect of ω-3 index on depression symptoms was detected in five of the eight models (see Table 18), in that ω-3 index predicted lower depression symptoms without the influence of the mediators. In addition, there was a significant total effect of the model ($b = -1.87$, $t(65) = -2.10$, $p = 0.04$), taking into account the direct and indirect effects of the model. Lastly, greater abundance of Lactobacillus significantly predicted higher levels of the inflammatory marker IL-8 ($b = 57.96$, $t(64) = 2.50$, $p = 0.02$).

**DHA (predictor) and BDI.** Two models were noted to show a significant direct effect of DHA on depression symptoms (see Table 19), where DHA predicted less depression symptoms, without taking into account the mediators. The total effect of DHA on depression symptoms was significant ($b = -2.40$, $t(65) = -1.97$, $p = 0.05$), taking the sum of the direct and indirect effects of the model. Additionally, one path coefficient was noted to be statistically significant in that greater Lactobacillus abundance significantly predicted higher levels of IL-8 ($b = 59.15$, $t(64) = 2.56$, $p = 0.01$).

**EPA (predictor) and BDI.** With EPA as the predictor, three path coefficients were statistically significant (see Table 20), similar to the results for trait-anxiety symptoms. In two
models, higher levels of EPA predicted higher levels of the inflammatory marker IL-6, once when Lactobacillus was a mediator ($b = 8.27$, $t(64) = 2.12$, $p = 0.04$) and when Bifidobacterium was a mediator ($b = 7.77$, $t(64) = 1.98$, $p = 0.05$). In addition, Lactobacillus significantly predicted higher levels of IL-8 ($b = 57.17$, $t(64) = 2.41$, $p = 0.02$).

**ALA (predictor) and BDI.** Only one path coefficient was significant (see Table 21) with ALA as the predictor; Lactobacillus was positively associated with inflammatory marker IL-8 ($b = 56.78$, $t(64) = 2.41$, $p = 0.02$).

**Summary for BDI.** Four ω-3FA variables (ω-3-index, DHA, EPA, ALA) were tested as predictors of depressive symptoms (BDI) in a total of 32 serial mediation models. Across all models, there was a significant total effect of ω-3 index and DHA predicting depressive symptoms. For five models when ω-3 index was the predictor, and two models when DHA was the predictor, there was a negative association with depression symptoms. There were some consistencies in the findings, specifically, when Lactobacillus and inflammatory markers IL-6 or TNF-α were mediators, a direct effect was noted. Collectively, across all 32 serial mediation models with depression symptoms as the outcome, Lactobacillus significantly predicted higher levels of inflammatory marker IL-8 and EPA significantly predicted higher levels of inflammatory marker IL-6.

**Global Summary of Mediation Analyses.**

Within the 128 serial mediation models conducted, several patterns were detected. When examining anxiety symptoms, there was a direct and total effect of ω-3 index and DHA predicting less trait-anxiety symptoms, however, there was no significant effect for state-anxiety symptoms. A significant total effect of ω-3 index and DHA on depression symptoms was noted
where \( \omega-3 \) index and DHA predicted fewer depression symptoms. Gut bacteria and inflammation were not found to mediate the relationship between \( \omega-3 \) intake and stress, anxiety, or depression symptoms. Consistently across all models, greater abundance of the gut bacteria Lactobacillus predicted higher levels of inflammatory marker IL-8 and EPA significantly predicted higher levels of inflammatory marker IL-6. Furthermore, inflammatory markers IL-1\( \beta \) and IL-6 were found to significantly predict more stress symptoms.
CHAPTER IV: DISCUSSION

The impact of nutrition on psychological health and cognitive functioning is a growing topic of interest among researchers (Gomez-Pinilla, 2008). As mental health disorders continue to rise globally, there is promising research supporting the importance of diet as a favorable therapeutic option for managing psychological well-being, specifically ω-3 FAs. It has been well established that ω-3 FAs are necessary for healthy mammalian central nervous system development (Innis, 1991; Perica & Delaš, 2011), and more recently have been recognized to play a favorable role on mental health symptoms (Golding et al., 2009; Ross, 2009).

With increasing interest in understanding the impact of ω-3 FAs on mental health outcomes, research has begun to examine the underlying mechanisms that may explain this relationship (Polokowski, Shakil, Carmichael, & Reigada, 2018). Much of the research conducted to date involve animal subjects with a great amount of heterogeneity in results. Two potential mediators based on the literature explaining the relationship between ω-3 FAs and mental health symptoms are the inflammatory response and gut bacteria (Kaplan, Rucklidge, Romijn, & McLeod, 2015). Specifically, it has been hypothesized that a poor-quality diet may promote alteration in the gut microbiome, leading to an immune response of increased levels of inflammatory markers, subsequently leading to depression (Berk et al., 2013). We contributed to this innovative and burgeoning field of research by examining the relationship between nutrition (along with its potential biological effect on gut bacteria and inflammation) with mental health outcomes.

This study examined the effect of ω-3 FAs directly and indirectly on mental health symptoms. The current study aimed to gain a better understanding of the ways in which ω-3 FAs, and potential mediators (e.g., gut bacteria, inflammation), are related to mental health outcomes.
among a sample of healthy human subjects. We hypothesized a full mediation model such that ω-3 FAs influence the gut microbiome by increasing healthy gut bacteria and consequently reducing inflammation, resulting in decreased stress, anxiety, and depressive symptoms.

The first aim of this study was to examine the cross-sectional relationships between ω-3 FAs, gut microbiome bacteria, inflammation, and stress, anxiety and depressive symptoms. Several significant correlations were detected across variables and are discussed below. The second aim of the study was to examine whether specific gut bacteria and inflammatory markers mediate the relationship between ω-3 intake and psychological outcomes. Given there are four outcome variables (stress, trait-anxiety, state-anxiety, depression), four predictor variables (ω-3 Index, DHA, EPA, ALA), and six mediator variables (Lactobacillus, Bifidobacterium, and TNF-α, IL-6, IL-8, IL-1β), 128 mediation analyses were conducted. Overall, across all serial mediation models, no mediation was detected. Thus, we did not find gut bacteria or inflammation to mediate the relationship between ω-3 FAs and stress, anxiety, or depression symptoms. However, several significant direct effects were noted within the models and are described below.

Omega-3 FAs and Mental Health Outcomes

One key finding from the present study is that ω-3 index and DHA were negatively associated with trait-anxiety and depressive symptoms. That is, for participants with greater amounts of ω-3 index or DHA, fewer depression and trait-anxiety symptoms were reported. Direct effects among these variables were also replicated in the mediation analyses. Our findings partially support our hypothesis that there would be a negative relationship between ω-3 FAs and stress, anxiety, and depressive symptoms. No relationships were noted between EPA or ALA and stress, anxiety, and depressive symptoms.
It is well established in the literature that higher levels of ω-3 FAs are associated with less depressive symptoms (Golding et al., 2009; Raeder et al., 2007) and more recently research has reported a similar association with anxiety disorders (Jacka et al., 2013), and comorbid depression and anxiety disorders (Thesing, Bot, Milaneschi, Giltay, & Penninx, 2017). Additional, support for the link between ω-3 FAs and mental health has also been tested using RCT designs. One RCT with 432 adult outpatients experiencing a major depressive episode demonstrated that participants taking ω-3 FAs showed reduced depressive symptoms as compared to those taking a placebo (Lesperance, 2011). Another RCT examining children with major depressive disorder who were administered a ω-3 supplement or placebo (Nemets, Apter, Bracha, & Belmaker 2006) found that ω-3 supplement demonstrated therapeutic benefits in depression based on both clinician and patient report. Furthermore, a RCT examining substance users found that ω-3 supplementation improved anxiety symptoms compared to those in a placebo condition (Buydens-Branchey, Branchey, & Hibbeln, 2008). Similarly, a systematic review and meta-analysis of ω-3 FAs and anxiety found much heterogeneity, yet an overall reduction in anxiety symptoms with those participants receiving ω-3 FA supplements compared to controls (Su, et al., 2018). The findings of this study further contribute to this growing body of research, supporting a negative association between ω-3 FAs and depression and anxiety symptoms.

While this study did find that ω-3 index and DHA levels were associated with fewer depression and trait-anxiety symptoms, no relationship was noted with the other psychological outcomes (state-anxiety or stress symptoms). Trait-anxiety and depression symptoms assessed in this study likely reflect a more stable, prominent, and consistent pattern of mood in participants which was negatively associated with ω-3 index and DHA levels. Furthermore, depression is
highly comorbid with anxiety disorders (Richter et al., 1998) which may explain the similar findings in trait-anxiety and depression symptoms. In comparison, state-anxiety can be described as an anxiety response to a specific stressor where a circumstance is perceived as threatening (Barnes, Harp, & Jung, 2002). Similar to state-anxiety, the PSS assessed stress symptoms over the past month (Cohen, Kamarck, & Mermelstein, 1994). The PSS and state-anxiety likely overlap as they are designed to assess perceived feelings of stress or anxiety in a more fluid context.

Others have also noted that more persistent and stable psychological symptoms are related to diet compared to more temporary states of anxiety or stress (Grosso et al. 2014a). The review paper conducted by Su and colleagues (2018) examining anxiety symptoms after ω-3 FA supplementation, supports our study findings as they note that subgroups with a clinical diagnosis, compared to those without, demonstrated a greater reduction in anxiety symptoms. Accordingly, diet may be a more beneficial intervention for populations with clinical symptoms rather than general populations. Clinical trials have shown that individuals with clinical diagnoses of major depressive disorder, anxiety, and early phase psychosis have benefitted from ω-3 FA supplementation (Chen, Chibnall, & Nasrallah, 2015; Grosso et al. 2014a; Su et al., 2018). In a cohort of French adults followed for two years, subjects whom consumed fatty fish or whom had an intake of ω-3 FA higher than 0.10% of energy intake demonstrated less risk of a depressive episode and of a recurrent depressive episode (Astorg, et al., 2008). These findings suggest that it may be more valuable to conduct future investigations of ω-3 FA supplementation with samples with more chronic symptomatology compared to individuals with sub-clinical mental health symptoms.
In addition, the current study found that the type of \( \omega-3 \) FA mattered. Results showed that DHA predicted less depression and trait-anxiety symptoms while EPA and ALA were not associated psychological outcomes. There has been some evidence supporting the negative relationship between DHA and depression symptoms in healthy adults (Mamalakis, Tornaritis, & Kafatos, 2002; Sarri, Linardakis, Tzanakis, & Kafatos, 2008). Specifically, it has been shown that higher DHA levels in mothers’ breast milk predicted lower rates of postpartum depression (Hibbeln, 2002). In a postmortem study, patients with major depressive disorder had significantly less DHA in the orbitofrontal cortex compared to controls (McNamara et al., 2007). Furthermore, one case control study investigating 800 United States military personnel compared to 800 controls, found that low levels of DHA was a significant risk factor for suicide, while there was no association with EPA levels (Lewis et al., 2011). These findings highlight the value of DHA. In particular, incorporating DHA via supplements or dietary sources may be a novel yet significant intervention when treating individuals with severe psychological symptoms such as major depression or suicidality.

One area that requires further examination is why our findings only showed an association between DHA and depression and trait-anxiety symptoms, but not other types of \( \omega-3 \) FA. Both DHA and EPA are found within the same food groups such as fish, shellfish, and algae (Anderson & Ma, 2009) which indicates that differences in dietary sources of our participants are an unlikely explanation. One possible explanation for the difference in \( \omega-3 \) FA type and psychological outcomes is that the process by which DHA and EPA metabolizes may be distinct from one another. Indeed, researchers have not clearly identified the ways in which DHA is metabolized. Across the literature, the majority of studies report DHA levels within the blood, however, it is unknown whether DHA supplementation truly increases the level of DHA in the
brain (Sublette, Ellis, Geant, & Mann, 2011). There seems to be varying mechanisms by which DHA is transported to the brain and to the blood based on animal models which remain to be unclear (Chouinard-Watkins, Lacombe, Metherel, Masoodi, & Bazinet, 2019). Furthermore, it has been established that there is greater abundance of DHA in the brain compared to EPA, however, it is unclear whether EPA metabolizes more quickly than DHA or if there is low EPA uptake by the brain (Sublette, Ellis, Geant, & Mann, 2011; Hallahan et al., 2016). Research has also demonstrated that DHA clears from plasma at a slower rate than EPA, with some evidence showing that after 24 weeks of discontinuing ω-3 supplements, DHA levels did not reach baseline levels (Arterburn, Hall, & Oken, 2006). Thus, DHA may be detected in red blood cells for longer periods of time than EPA. In regards to the present study, we found support for DHA, and not EPA, predicting less depression and trait-anxiety symptoms based on levels within whole blood samples. This finding further supports the growing acknowledgement that there are differences between EPA and DHA. When evaluating the literature examining EPA and DHA, it is important to evaluate RCT’s and cross-sectional research independently. For instance, much of the research trials administering EPA and DHA supplements to participants do not objectively assess EPA and DHA in participants (Hallahan et al., 2016) and many of the cross-sectional studies do not assess EPA and DHA intake via diet or supplements.

Given that the present study examines cross-sectional data, it is necessary to consider that individuals with less anxiety and depression may make healthier food choices that results in higher ω-3 FA intake rather than their diet affecting their mental health. For instance, undergraduate students who reported increased stress while waiting for an exam grade experienced increased tendency to eat compared to a control group (Macht, Haupt, & Ellgring, 2004). Furthermore, women who are in stressful situations tend to consume larger quantities of
food compared to women in a low-stress condition (Habhab, Sheldon, & Loeb, 2008). Research has also shown that foods higher in fat may reduce stress (Finger, Dinan, & Cryan, 2011; Maniam & Morris, 2010). Careful consideration must be made when evaluating the relationship between diet and mental health. There is likely a bidirectional relationship between affect and food choice in which psychological state influence food choice and food consumed can influence affect (Gibson, 2006).

Omega-3 FAs and Inflammation

Contrary to much of the literature and our hypothesis, lower ω-3 FA intake was not associated with lower inflammatory markers. In general, there was no relationship between ω-3 FAs and inflammatory markers, with one exception, higher levels of EPA significantly predicted greater levels of inflammatory marker IL-6. Some clinical trials administering ω-3 supplements in medical populations (e.g., hypertriglyceridemia, obesity) have not found an effect of ω-3 FAs on inflammation, which may be due to treatment duration or negative impacts on insulin sensitivity which can increase inflammatory markers (Chan, Watts, Barrett, Beilin, & Mori, 2002; Skulas-Ray et al., 2011). However, our findings were unexpected given that ω-3 FA supplementation has been widely shown to reduce inflammatory markers (Li, Huang, Zheng, Wu, & Li, 2014; Wall, Ross, Fitzgerald, & Stanton, 2010). One study examining the impact of EPA and DHA on inflammatory markers in adipocytes (cells storing fat) has shown that EPA supplementation increased the concentration of IL-6 while DHA decreased the concentration of IL-6 (Prostek, Gajewska, Kamola, & Balaninska, 2014). The authors posit that EPA and DHA may have a different effect on the inflammatory process during various stages of cell maturation which may explain why EPA has shown to potentially both reduce (Hao et al., 2010) and increase inflammatory markers. Although, IL-6 is largely conceptualized as having pro-
inflammatory properties, a couple of review articles (Hawkley, Bosch, Engeland, Cacioppo, & Marucha, 2007; Woods, Vieira, & Keylock, 2009) note the anti-inflammatory nature of IL-6 which may be one possible explanation as to why EPA may be related to increased IL-6. It has been found that IL-6 may inhibit the response of increased levels of IL-1β and TNF-α and play a role in impeding the inflammatory response (Luheshi et al., 1997).

It is important to acknowledge that the method of inflammatory marker assessment was via saliva. Salivary inflammatory markers have been shown to be sensitive to acute stress, even collecting salivary samples at multiple time points at one laboratory visit can demonstrate differences in levels of inflammation (Slavish, Graham-Engeland, Smyth, & Engeland, 2015). In the present study, we attempted to control for experimental stress effects on inflammation by collecting the saliva sample at the beginning of the lab visit; prior to the finger prick and the administration of self-report measures to reduce the potential contribution of acute stress in salivary measurement. Additionally, we also attempted to control for confounding inflammation factors by excluding participants from our study who were diagnosed with an inflammatory or endocrine illness, engaged in heavy alcohol use (more than two glasses of alcohol per day) or heavy smoking (more than eight cigarettes per day). However, we did not control for the waketime of participants. It has been shown that inflammatory markers may vary in salivary samples based on time of the day a sample is provided (DeSantis et al., 2013). Lastly, oral health status (e.g., gingivitis) may confound measures of salivary inflammation (Deinzer et al., 2004) which was not assessed in our sample.

**Inflammation and Mental Health Outcomes**

We hypothesized that higher levels of inflammation would be associated with more stress, anxiety, and depressive symptoms. This was partially supported as there was a positive
relationship noted between inflammatory marker IL-1β and depression symptoms, and higher levels of IL-1β and IL-6 significantly predict higher levels of stress symptoms. These findings are consistent with a vast majority of the literature supporting the positive association between inflammation and anxiety and depression (da Silva et al., 2017; Das, 2016; Dowlati et al., 2010; Howren, Lamkin, Suls, 2009).

These findings add to the robust literature that inflammation is a critical component of psychological health and should be targeted while treating patients with psychiatric illnesses. A meta-analysis examining 14 RCTs with over 6,000 patients concluded that anti-inflammatory treatment, specifically, nonsteroidal anti-inflammatory drugs, reduced depression symptoms and did not increase patient’s risk for adverse events (Köhler, et al., 2014). Furthermore, there is much evidence supporting that stress activates an inflammatory response via the hypothalamic-pituitary-adrenal (HPA) axis which increases the expression of pro-inflammatory cytokines (Liu, Wang, & Jiang, 2017). There is considerable evidence for the bidirectional relationship between inflammation and depression, however it is likely that inflammation does not play a role in all individuals with depression. Keicolt-Glaser and colleagues (2015) discuss the ways in which multiple factors may contribute to a more robust inflammatory response (e.g., childhood adversity, obesity, poor diet) in some individuals with depression and that targeting inflammation in this population is essential for successful treatment.

**Gut Bacteria and Inflammation**

Although not hypothesized *a priori*, there was a small positive association between Lactobacillus and IL-8 in that increased levels of Lactobacillus significantly predicted higher levels of IL-8. This finding was unanticipated as Lactobacillus and Bifidobacterium were hypothesized to have anti-inflammatory effects. If Lactobacillus does in fact promote
inflammation, this would be an important finding to replicate given that this is classified as a healthy bacteria and incorporated within many probiotic supplements. Similar to the present finding, one study unexpectedly found that a high dose probiotic, including Lactobacillus did increase IL-8 production (Zhang, Li, Caicedo, & Neu, 2005). The authors posit that the overgrowth of some bacteria may potentially stimulate the inflammatory response. Thus, it is possible that our sample had high levels of Lactobacillus which may have increased IL-8 production.

The research is novel when examining the relationship between gut bacteria and inflammatory markers, with much of the studies conducted in animal models. A study examining the effect of Lactobacillus in arthritic rats found that the rats that orally consumed Lactobacillus experienced less joint inflammation than those rats who did not consume Lactobacillus (Baharav, Mor, Halpern, & Weinberger, 2004). One study conducted in human subjects found that bacteria Faecalibacterium prausnitzii, has been shown to demonstrate anti-inflammatory effects in patients with Crohn’s disease, specifically blocking IL-8 production (Sokol et al., 2008). Schirmer and colleagues (2016) have developed a database of predicted relationships between gut bacteria and cytokine responses based on 500 healthy individuals. They note a negative association between gut bacteria, Bifidobacterium adolescentis and TNF-α production. Due to the inherent difficulty of studying the gut microbiome (e.g., multiple confounding variables), clear relationships between gut bacteria and inflammatory markers are challenging to assess. As research continues to develop and the techniques become more advanced, investigators will be able to more accurately link specific gut bacteria to physiological and potentially psychological outcomes.
**Strengths and Limitations**

The present study is novel in that it is the first to study the associations between ω-3 intake, gut bacteria, inflammatory markers, and anxiety and depression outcomes in a human sample. We found evidence that higher levels of ω-3 index and DHA were associated with lower reported trait-anxiety symptoms, depressive symptoms, and lower concentrations of the inflammatory marker IL-1β. We aimed to understand the ways in which ω-3 FAs impact mental health outcomes by examining potential mediators. While gut bacteria and inflammation were not found to mediate the relationship between ω-3 and psychological outcomes, this study can be utilized as a framework and set the foundation for future research to examine these variables.

It has been recognized that one major source of heterogeneity in ω-3 FA research is the influence of sex (Martins, 2009). In addition, many studies do not report the breakdown of participant sex which is problematic. One strength of the current study is that, our sample was comprised of all female participants. Overall, females have been found to have higher levels of DHA compared to males, with the role of estrogen as one explanation (Kitson, Stroud, & Stark, 2010). Estrogen has been shown to cause higher concentrations of DHA in women than in men (Giltay, Gooren, Toorians, Katan, & Zock, 2004). Kitson and colleagues (2010) posit that the difference in DHA levels between males and females may be evolutionary; females have higher concentrations of DHA in order to provide adequate DHA for fetal development. Thus, examining the impact of ω-3 supplementation in men and women separately may help explain some of the variance in findings.

Another strength of the current study was the utilization of dried blood spot samples to assess ω-3 levels in our participants. This allows for an objective measure of ω-3 FAs rather than calculating ω-3 intake based on subject self-report measures such as food frequency
questionnaires. Based on our procedures of measuring ω-3 index via dried blood spots, we obtained fatty acid values from the whole blood fatty acid profile, meaning both red blood cells and plasma are collected by a finger prick. This method of assessing fatty acids via whole blood spot collection has been found to be reliable, non-invasive, and more economical compared to methods that require centrifugation (Gordon Bell et al., 2011). One study utilizing whole blood fatty acid demonstrated increases in EPA and DHA levels six weeks after a dietary intervention (Rise, Colombo, Ghezzi, Mauro, & Orlandini, 2011). Furthermore, four weeks post-intervention, DHA levels continues to increase and EPA levels decreased slightly. These findings support the utilization of whole blood fatty acid profiles as a method to assess ω-3 FA levels.

One strength, and also a potential study limitation, is the ethnic diversity of the sample. The study was conducted in a metropolitan city in an urban university setting with a diverse sample of approximately 25% Black, 25% White, 25% Hispanic/Latino, 15% Asian, and the remaining participants identified as Mixed or Other. It is important to examine this demographic feature when studying ω-3 FAs given that ethnicity does seem to impact accessibility to ω-3 FAs or may influence genetic processing of ω-3 FAs. Certain cultures are exposed to higher concentrations of ω-3 FAs such as Inuit/Eskimo and Japanese populations (Patel, Tracey, Hughes, & Lip, 2010). Furthermore, some Europeans such as Italians tend to more efficiently incorporate ω-3 FAs into their diet (Patel, Tracey, Hughes, & Lip, 2010). As described in our results, Asian and Black participants were found to have higher levels of ω-3 index and DHA than other races, however this was anticipated based on the literature (Villegas, Takata, Murff, & Blot, 2016; Xiao et al., 2016).

A main limitation of this study is the methodological use of cross-sectional data which prevents us from drawing robust conclusions since we are unable to determine the temporal order
of the relationships analyzed. Based on the current state of the research, we hypothesized that ω-3 FAs will influence the gut microbiome by increasing healthy gut bacteria and subsequently reducing inflammatory cytokines, leading to decreased anxiety and depressive symptoms. However, this hypothesized theoretical framework may not be accurate. One alternative possibility is that anxiety and depressive symptoms may be increasing inflammation, leading to reductions in healthy gut bacteria with ω-3 FAs moderating this relationship. For instance, individuals with anxiety or depressive symptoms may demonstrate higher levels of inflammatory markers and lower levels of healthy gut bacteria, however, consuming higher amounts of ω-3 FAs may serve as a protective factor against these negative effects.

Furthermore, the examination of ω-3 FAs alone may be limited. Researchers have posited that it is important to examine both ω-3 and ω-6 FAs, as together they play a role in inflammatory responses in the human body (Wall, Ross, Fitzgerald, & Stanton, 2010). However, there is controversy within the literature regarding the utilization of the ω-6/ω-3 ratio. Much research has been done surrounding ω-3 FA and their anti-inflammatory properties however the impact of ω-6 FAs is not as straightforward. Some ω-6 FAs have anti-inflammatory properties (e.g., dihomo-γ-linolenic acid) while others promote inflammation (e.g., arachidonic acid) (Harris et al., 2009; Poorani, Bhatt, Dwarakanath, & Das, 2015). Over the past century, there has been a drastic shift in dietary patterns, with a substantial increase in the amount of ω-6 FAs consumed, due to the large amount of LA-enriched vegetable oils in the food supply, and subsequently a deficiency of ω-3 FAs in the Western diet (Simopoulos, 2002). Some researchers support the belief that this imbalance has resulted in a high ω-6 to ω-3 ratio of approximately 16:1, fostering the development of various diseases such as cardiovascular disease and note that
the ideal ratio of ω-6 to ω-3 is 4:1 or 1:1 (Simopoulos, 2008). However, others claim that there is no evidence that reducing ω-6 FA intake will prevent chronic illness (Harris, 2006).

Another weakness to this study lies within preliminary assessment and examination of gut microbiome data in human subjects. There is more to be learned in the sampling and processing of stool samples and recent research is attempting to create guidelines for collecting stool samples to improve the validity of gut microbiome results (Gorzelak et al., 2015). There is a limited understanding of the ways in which to interpret the gut microbiome as there are countless numbers of microorganisms in the gastrointestinal tract. It is difficult to parse out the specific impact of a particular bacteria given the all the factors that influence the microbiome. Various environmental factors can influence the gut microbiome including antibiotics, diet, clean environments, and stress (Kaplan, Rucklidge, Romijn, & McLeod, 2015). Some literature has supported the idea that more diversity in the microbiome is healthier and having a less diverse microbiome may be associated with chronic medical conditions (Liu, 2017). Thus, the gut bacteria findings in the present study are preliminary and must be interpreted with caution.

A methodological limitation of the present study was the adherence of participants to complete the uBiome gut kits. Participants completed majority of the study assessments in the research lab, however, they subsequently were asked to submit a stool sample using the uBiome kit after the lab visit. Of the 79 total participants, 67 completed and mailed in the uBiome kit which is an 84.8% adherence rate. This resulted in missing gut bacteria data for a portion of our sample. On average, our participants mailed out the uBiome kits within 4 days of receiving the kit and completing baseline measures. In an attempt to increase adherence rates, research assistants sent an email to participants the day after their assessment to verify their status on submitting the kit. Additionally, since the uBiome kit was completed outside of the laboratory, it
is unclear whether the participants correctly adhered to the instructions given to complete the stool sample kit. There is little research published on adherence rates of gut bacteria kits provided to study participants in a lab setting as this is a novel assessment in human participants. To date, studies have collected fecal samples on human subjects via a research nurse collecting directly from an adult participant’s home (Hill et al., 2016) or have been collected in infant samples while they are admitted to the hospital (Williams et al., 2019). There has been some research on adherence rates for self-collection methods in the cancer screening literature. When offered to self-screen for cervical cancer at home with a kit, response rates were approximately 20-25% for women who received a kit in the mail (Gupta et al., 2018). Additional research is needed surrounding adherence rates for self-collection procedures of stool samples by participants outside of laboratory settings.

Another limitation was that our data did not meet the assumptions for normality. Due to the non-normality of the data, Spearman’s correlation was utilized for the first aim of the study. Spearman’s correlation is recommended when analyzing non-normal distributions while maintaining Type 1 error rates (Bishara & Hittner, 2012). Additionally, as with any research study using self-report data, there is a risk for biased responses. We administered self-report measures of stress, anxiety, and depression symptoms. It is possible that participants were inclined to respond to measures in a socially desirable manner (van de Mortel, 2008) and conducting a structured interview to assess psychological symptoms could have potentially improved the accuracy of mental health symptoms reported.

**Future Directions**

Based on the synthesis of the current research, a recommended next step would be to conduct RCTs investigating various formulations of ω-3 supplementation within individuals with
subthreshold and clinical levels of depression and anxiety. Prospective longitudinal studies such as these will produce findings that can clearly be interpreted as causal, and will reveal the magnitude of the specific types of ω-3 FAs on different types of symptomatology. Incorporating repeated measures in clinical trials are a critical component to assess changes over time. Moreover, prospective studies with a larger sample can test whether additional endogenous variables moderate the relationship between ω-3 intake and psychological symptoms such as race, gender, and biological markers (e.g., inflammatory markers, hormones). Future studies can also improve the assessment of inflammation in particular by utilizing blood samples over saliva. Measuring inflammatory markers via saliva has some benefits such as being non-invasive and minimizing participant burden, however, there are methodological downfalls such as inconsistency in collection methods and large variability in cortisol concentrations (Desai & Mathews, 2014).

As mentioned in the Introduction, there are no established guidelines of ω-3 dosages to improve mental health symptoms. Across studies, there is a large amount of variability in the measurement and administration of ω-3 FAs. These methodological flaws seem to have an impact on the results reported in the literature. For instance, a meta-analysis assessing the effects of ω-3 FAs on major depressive disorder deemed the studies to be poor quality (e.g., non-blinding of participants to ω-3 supplement conditions, small sample sizes) and noted that there was a large amount of heterogeneity among the studies reviewed (Appleton, Sallis, Perry, Ness, & Churchill, 2015). Additionally, there is a large variety in the dosage, purity, and type of ω-3 FA supplements administered in clinical trials. As such, while conducting their meta-analysis, Grosso and colleagues (2014) created categories of the purity of supplements (e.g., mainly EPA, pure EPA). In addition, the researchers noted a widespread length of time in the trials from 4
weeks to 160 weeks. In addition, each type of ω-3 (DHA, EPA, ALA) has shown to demonstrate differing effects on biological processes (Dyall, 2015), supporting the notion that standardization of ω-3 supplements administered in research trials is essential. The inconsistency across methods is a critical weakness within the literature, thus, it is challenging to evaluate the literature and to infer any robust conclusions between ω-3 FA and mental health outcomes. Thus, it is necessary for researchers to develop standard recommendations across ω-3 types, dosages, and durations within clinical trials in order to productively move forward in this field of research.

There is continually a demand to improve assessment methods when conducting research, particularly within nutrition research. Many researchers utilize food frequency questionnaires, food diaries, and 24-hour recall methods to assess diet. Dietary self-report measures have consistently shown to result in underreporting of energy intake (Freedman et al., 2014) and are inaccurate due to poor evaluation of portion size or have an incomplete list of foods (Thompson & Subar, 2017). Incorporating the use of objective assessment measures such as blood samples (Johnston, Deuster, Harris, Macrae, & Dretsch, 2013) can improve accuracy in nutritional assessments and avoid participant biases in diet recall methods. Additionally, it would be beneficial for researchers to objectively assess both ω-6 and ω-3 FA levels. As mentioned earlier, there is controversy regarding the significance of the ω-6 to ω-3 ratio, however many studies only assess ω-3 FA levels. Including the assessment of various ω-6 FAs would increase our understanding of the role of ω-6 FAs on physical and mental health outcomes.

Examining ethnicity more rigorously within ω-3 research is necessary given the clear baseline differences in ω-3 levels across races. Our study found higher levels of ω-3 levels in participants that identified as Asian/Pacific Islander and Black, compared to White and Hispanic participants. These variations have been identified in the literature (Villegas, Takata, Murff, &
It is also been noted that individuals of Japanese descent have much higher concentrations of ω-3 levels compared to Americans (Iso et al., 1989). It is important for researchers to gather an accurate assessment of racial/cultural background, dietary history, and regional location of their participants prior to enrollment in a clinical trial in order to stratify their sample appropriately. This will increase the likelihood of well-defined results and a clearer understanding of the implications of ω-3 supplementation. Furthermore, given the differences in ω-3 levels across racial backgrounds, there are likely varying needs for different races. For instance, one review of nearly 300 studies evaluated EPA and DHA levels across countries globally (Stark, Van Elswyk, Higgins, Weatherford, & Salem, 2016). The authors identified a large amount of variability in ω-3 levels across regions, with those in the very low range potentially having an increased risk of chronic illness. Thus, ω-3 recommendations may not be consistent across racial groups and those with lower levels ω-3 will likely require higher ω-3 dosages.

Based on our current findings, a next phase of research would be to develop an RCT investigating the impact of different types of ω-3 supplementation, such as, EPA versus DHA predominant formulas, across individuals with varying degrees of clinical symptoms, such as, subthreshold versus clinical levels of depression and anxiety. Utilizing diagnostic clinical interviews to evaluate the severity of psychological symptoms would be ideal. It would be important to stratify the sample by racial background due to variances across ω-3 FA levels. Another option would be to create a less ethnically diverse sample to help remove any confounding racial effects. Moreover, with the RCT, it will be necessary to utilize objective measures of ω-3 assessment (e.g., blood samples) to eliminate the potential of underreporting via self-report measures. Measuring ω-3 levels at multiple time-points (e.g., baseline, mid-
assessment, post-assessment, follow-up) would also shed light on the ways in which ω-3 FAs metabolize and how long ω-3 FAs remain within the human body.

Additional work needs to be done within psychological nutrition research to improve the standardization across trials. Specific recommendations have been suggested including developing consistency across the dosage and duration of ω-3 supplements administered, improving the measurement of diet and psychological symptoms, increasing diversity within samples, and designing clinical trials that test mediation (Polokowski, Shakil, Carmichael, & Reigada, 2018). These are some strategies to gain better insight into the ways in which ω-3 FAs may be related to psychological symptoms.

**Clinical Implications**

As rates of mental health disorders increase, it is necessary to evaluate lifestyle factors that may impact these disorders, as these factors have been overlooked in psychological intervention research. Nutrition has been identified as one avenue of research that relates to multiple biological mechanisms which may in turn influence anxiety and depression. Implementing dietary assessment and interventions into evidence-based practices can be cost-effective and more easily tolerated than psychotropic medications (Freeman et al., 2006; Mozaffarian & Rimm, 2006). With increased attention focused on the connection between nutrition and mental health, there is promising research supporting the importance of diet as a favorable therapeutic option for managing psychological well-being.

**Assessing Diet in Clinical Practice**

Although nutritional status has been identified as an important component to mental health, clinicians are not provided standardized training to assess diet in their patients and therefore do not routinely inquire about their patient’s dietary intake (Burks & Keeley, 1989;
Terry & Reeves, 2015). The biopsychosocial framework is a valuable model when intervening with diet because it considers the biological, social, psychological, and environmental factors associated with health behaviors (Suls & Rothman, 2004). Furthermore, with the expansion of multidisciplinary teams, clinicians will have increased access to dieticians or nutritionists to consult with or make referrals when they need additional support surrounding patients’ dietary issues.

There are multiple ways to evaluate diet in the context of mental health care including assessing food selection, supplementation, or deficiencies (Walsh, 2011). Conducting a basic assessment of a patient’s diet may help guide treatment recommendations. For instance, caffeine has shown to increase anxiety and psychotic symptoms and impact sleep, thus a simple assessment of caffeine intake is recommended as part of any psychiatric assessment (Winston, Hardwick, Jaberí, 2005). It is also suggested that clinicians inquire about nutritional supplements, as many patients assume they are harmless, yet they have the potential to negatively interact with pharmaceutical drugs (Singh, 2005). With a brief assessment of diet, mental health professionals have the opportunity to discover key features of their patients which may influence treatment recommendations.

**Incorporating Dietary Interventions into Clinical Practice**

Dietary and nutritional supplements have been explored in relation to psychological health. For instance, a review article reported that low levels of folate (a B-vitamin) and vitamin B-12, have been found in samples of depressed patients (Coppen & Bolander-Gouaille, 2005). Furthermore, depressed patients responded better to antidepressant medication when folate and vitamin B-12 levels were higher (Coppen & Bolander-Gouaille, 2005; Miller, 2008). A recent meta-analysis examining the efficacy of adding nutritional supplements to antidepressant
concluded that overall results are inconclusive due to mixed results across studies, however there is some support for the supplementation of zinc and ω-3 FAs (Schefft, Kilarski, Bschor, Kohler, 2017). Thus, some of the literature supports the notion that adding supplements to established therapeutic practices may increase efficacy, with ω-3 FAs in particular showing benefit (Dome, Tombor, Lazary, Gonda, & Rihmer, 2019).

There is evidence that consuming ω-3 FAs via diet (e.g., salmon, enriched yogurt) demonstrate increased levels of plasma EPA and DHA (Elvevoll et al., 2006; Rise, Colombo, Ghezzi, Mauro, & Orlandini, 2011) compared to taking an ω-3 FA supplement (Ratnayake & Galli, 2009). Additionally, there is some evidence that consuming an ω-3 FA supplement with a meal high in fat may improve absorption of fatty acids (Opperman, 2013). Accordingly, if it is feasible for patients, it is recommended to consume ω-3 FAs via dietary intake as it seems this method has more advantageous effects compared to supplementation alone. Based on our findings, DHA in particular seemed to be related to psychological outcomes. Thus, it is recommended that foods higher in DHA such as seafood be incorporated into a patient’s treatment plan may be a simple yet effective intervention. Additionally, based on the present study, it seems that practical applications should aim to target more severe clinical samples, as they demonstrate more benefit from ω-3 FA supplementation.

Common Barriers in Dietary Interventions.

It is known that individuals with higher rates of anxiety and depressive symptoms generally have poorer lifestyle behaviors (e.g., poorer diets) which may have influenced our findings (Parletta, Aljeesh, and Baune, 2016), for instance, they would be less likely to consume ω-3 FAs or diets that may increase healthy gut bacteria. In practice, there are multiple factors to consider when investigating and intervening with dietary habits including access to food, social
economic status, nutritional education, living environments and cultural values. It has been found that individuals with a lower socioeconomic status are exposed to poorer quality food options which results in unhealth eating patterns (Ford & Dzewaltowski, 2008). Additionally, individuals are consuming more calories than ever before due to our culture being flooded by fast food restaurants, larger portion sizes, and an increase in energy dense foods, all for a low cost (Drewnowski & Darmon, 2005). Results from a meta-analysis show that on average, eating a healthier diet, such as fruits, vegetables, fish, and nuts, is more expensive than consuming a less healthy diet consisting of meats and refined grains (Rao, Afshin, Singh, & Mozaffarian, 2013). Many patients may not have the means or accessibility to access certain food options. Thus, clinicians may face larger challenges when attempting to provide dietary recommendations and must consider social and environmental factors.

Although significant work is still needed, developing clinical interventions that incorporate a dietary component may be a noteworthy next step in treating psychological conditions.

Conclusion

As there are drastic shifts in diets across the globe, the literature continues to identify trends in how diet is associated with mental health outcomes. Specifically, ω-3 FAs possess numerous physical and mental health benefits and are acknowledged as a necessary component of a healthy lifestyle. Recently, there has been an increase in research investigating potential biological mechanisms that may clarify the ways in which ω-3 FAs may prevent or treat mental health symptoms. Furthermore, growing evidence suggests that the gut microbiome promotes or prevents inflammatory responses which in turn may lead to neuropsychiatric disorders. The present study aimed to expand empirical efforts to explore the utility of ω-3 fatty acids by
gaining a greater understanding of the impact on inflammation, gut bacteria, and mental health outcomes.

Main findings from the current study demonstrated that higher levels of ω-3 index and DHA significantly predicted fewer trait-anxiety and fewer depressive symptoms suggesting that ω-3 supplementation may demonstrate benefit over mental health symptoms. Moreover, higher levels of inflammatory markers IL-1β and IL-6 were shown to significantly predict higher levels of stress symptoms, while higher levels of EPA significantly predicted higher levels of IL-6. Lastly, greater abundance of gut bacteria Lactobacillus significantly predicted higher levels of IL-8. We are in the preliminary stages of understanding the ways in which specific nutritional components such as ω-3 FAs, may have an impact on psychological health, yet researchers must improve the methodology examining these variables in order to develop meaningful conclusions. In order to utilize ω-3 FAs as therapeutic option for depression and anxiety, we must first understand the mechanisms by which they operate to develop the most effective treatment options.
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Acronyms

ALA: α-linolenic acid
AA: Arachidonic acid
BDI: Beck Depression Inventory
CRP: C-reactive protein
DHQ: Diet History Questionnaire
DHA: Docosahexaenoic acid
EFA: Essential Fatty Acid
EPA: Eicosapentaenoic acid
GIT: Gastrointestinal tract
GAD: Generalized Anxiety Disorder
ILD-6: Interleukin 6
IL-8: Interleukin 8
IL-1b: Interleukin 1 beta
LA: Linoleic acid
MDD: Major Depressive Disorder
MeSH: Medical subject headings
OCD: Obsessive-Compulsive Disorder
ω-3 FAs: Omega-3 fatty acids
ω-6 FAs: Omega-6 fatty acids
PSS: Perceived Stress Scale
TNF-α: Tumor necrosis factor alpha
STAI: State-Trait Anxiety Inventory
THE CITY UNIVERSITY OF NEW YORK

Brooklyn College

Department of Psychology

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

**Title of Research Study:** The Relationship between Diet, Inflammation, Gut Flora and Mental Health

**Principal Investigator:** Ashley Polokowski, M.A.
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**Faculty Advisor:** Laura Reigada, Ph.D.
Assistant Professor
Brooklyn College
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Brooklyn, NY, 11210
718-951-5000 ext. 6021

**Research Sponsor:** Schwab Donor Advised Fund

You are being invited to participate in a research study because you are at least 18 years of age, are not pregnant, have not been diagnosed with inflammatory or endocrine disorders, have not been diagnosed with any psychiatric disorders, do not drink or smoke excessively, do not use psychoactive drugs, do not have a history of drug abuse, have not been in treatment (psychotherapy and/or medication) for anxiety less than 6 months, and you have notified the researchers of any food allergies you have, particularly to fish.

**Purpose:**

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Appendices

Appendix A. Informed Consent
The purpose of this research study is to understand the relationship between the bacteria present in our gut, inflammation, the food we eat, and our mood. The results of this study will show how these elements are connected and how imbalances in some elements could influence the others.

**Procedures:**
If you volunteer to participate in this research study, we will ask you to do the following:

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<th>Time</th>
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| **Pre-test** (Day 0) | Brooklyn College Psychology Lab, James Hall, Room 4611 or Room 5309, 2900 Bedford Ave, Brooklyn, NY (“REACH Lab”) | Meet with Principal Investigator (PI) or Research Personnel  
Complete multiple questionnaires:  
1. Demographic Questionnaire (e.g., age, sex, ethnicity)  
2. Diet History Questionnaire (DHQ): assesses regular eating habits  
3. State-Trait Anxiety Inventory (STAI): evaluates anxiety symptoms  
4. Beck Depression Inventory (BDI): evaluates depressive symptoms  
5. Perceived Stress Scale (PSS): evaluates stress symptoms  
• Provide a saliva sample  
• Receive a stool sampling kit and directions on collecting stool samples and submitting kit to PI or mailing to external laboratory  
• Receive supply of omega-3 fatty acid pills, probiotic, or combination of both, and directions on taking the supplements  
• Receive directions on diet and lifestyle habits to avoid or to note during experiment  
• Receive directions on recording omega-3 and omega-6 content throughout the week |
| **Pre-test** (Day 0) | In private | In private collect own stool sample and follow directions to submit to external lab via mail, or return kit to research staff to submit via mail |
| **Experiment** (Week 1-Week 12) | In private | Take supplements daily as directed  
Record diet once per week |
| **Post-test** (Week 13) | REACH Lab | Return to REACH Lab to meet with PI  
Complete multiple questionnaires:  
1. Demographic Questionnaire  
2. Diet History Questionnaire (DHQ)  
3. State-Trait Anxiety Inventory (STAI)  
4. Beck Depression Inventory (BDI)  
5. Perceived Stress Scale (PSS) |
- Provide a saliva sample
- Receive a stool sampling kit and directions on collecting stool samples and submitting kit to PI or mailing to external laboratory

<table>
<thead>
<tr>
<th>Post-test (Week 13)</th>
<th>Debriefing</th>
<th>In private</th>
<th>In private collect own stool sample and follow directions to submit to outside lab via mail, or return kit to research staff to submit via mail</th>
</tr>
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**Time Commitment:**
Your participation in this research study is expected to last for a total of 13 weeks.

**Potential Risks or Discomforts:**
- The study involves minimal risk for breach of confidentiality.

- **For Questionnaires:** If the sensitive nature of some of the questions regarding mood causes you distress and you would like to speak to a mental health professional, or you would like to obtain mental health services for any reason, please contact the Brooklyn College Personal Counseling Center in room 0203 in James Hall, 2900 Bedford Ave, Brooklyn, NY or call them at 718-951-5363. Additionally, a Suicide Prevention hotline can also be contacted 24 hours a day at 1-800-273-8255. Alternatively, call 911 or go to the nearest hospital emergency room if you believe that you may be in danger of harming yourself or anyone else.

- **For stool sample:** You may feel uncomfortable providing a stool sample. The researchers have provided you an individual kit with directions which you can use to collect your stool in private. You have the option of mailing out your sample via mail to uBiome, the stool testing service, yourself or can give your individual kit to the research staff to mail out. All personal information provided them will remain confidential and you will be provided the privacy practices of uBiome upon request.

- **For taking Supplements:**
  - Consuming omega-3 fatty acid supplements, or fish oil pills, have generally been found to be safe but people in previous studies have reported side effects such as burping and a mild fishy aftertaste, or diarrhea. Taking capsules with meals minimizes these side effects. Omega-3 fatty acids may become toxic if oxidized, but this can be prevented by refrigerating the pills before consumption. Although not found as dangerous, omega-3s may also impact your cholesterol and triglyceride levels, which you may want to monitor with your doctor.
  - Probiotics are generally regarded as safe and can be found naturally in dairy and fermented products such as yogurt. Although very rare, probiotics possess the risk of causing bacteria in the blood and inflammation of the inner heart lining especially in patients with impaired immune systems.
**Potential Benefits:**
- This study will help you gain essential dietary nutrients recommended by the American Heart Association and the Federal Government.
  - Studies suggest that consumption of omega-3 fatty acids could improve cholesterol profiles of people by increasing HDL and decreasing LDL and could decrease your risk of breast cancer. Studies have also shown benefits of omega-3 fatty acids for people with cardiovascular disease, Type II Diabetes Mellitus, and Rheumatoid Arthritis.
  - Studies also suggest that consumption of probiotics have beneficial effects and could potentially lower cholesterol, hypertension, and postmenopausal symptoms, prevent diarrhea, and might even help people with lactose intolerance or other food allergies.
  - Lastly, your participation will also benefit a new area of research by helping us understand how what we eat can affect our mood.

**Alternatives to Participation:**
The alternative to study participation is not to participate. You may wish to consult your primary care physician before participating. Aside from compensation for participating you will not be reimbursed for visiting a doctor and it is your responsibility to speak with your doctor if you decide to do so. You have the option not to participate in the study.

**Costs**
All program-related costs (e.g., supplement pills) associated with this study, with the exception of travel expenses to the lab, will be provided to you.

**Payment for Participation:**
To compensate you for the time spent at the pre- and post-test, you will receive $25 at both time points. No reimbursement will be provided if the final assessment is not completed.

**Research Related Injury**
In the event of injury resulting from your participation in this research study, the facilities at Brooklyn College and professional attention will be made available to you at your expense. Financial compensation from Brooklyn College will not be provided. If you believe that you have suffered an injury related to this research as a participant in this study, you should contact the research staff.

**New Information:**
You will be notified about any new information regarding this study that may affect your willingness to participate in a timely manner.

**Confidentiality:**
We will make our best efforts to maintain confidentiality of any information that is collected during this research study, and that can identify you. We will disclose this information only with your permission or as required by law.
We will protect your confidentiality by storing all identifying information (name and email) in a password-protected file saved on a study flash drive stored in a locked office. Identifying information will be kept separate from all other study data to ensure confidentiality. Additionally, all questionnaires, saliva samples, computer tasks and worksheets will only include your participant ID and de-identified paper forms will be stored in a cabinet in a locked office. The list with identifying information will be destroyed upon completion of data collection. Consent forms with names will be stored separately in a locked office, will not include any linking information (i.e., ID number) and will be destroyed after 3 years.

You have the option to either directly mail their fecal samples to uBiome or to have the research team mail them on your behalf. The research team will not store any fecal samples and will dispose of any extras that you may provide. Any personal health information that you provide will be protected by the uBiome privacy practices; uBiome is required by the Health Insurance Portability and Accountability Act of 1996 (HIPAA) to keep all health information confidential.

If the investigators learn that you or someone with whom you are involved is in serious danger of potential severe harm, they may need to warn those who are in danger and contact other agencies to ensure safety.

The research team, authorized CUNY staff, the research sponsor, and government agencies that oversee this type of research may have access to research data and records in order to monitor the research. Research records provided to authorized, non-CUNY individuals will not contain identifiable information about you. Publications and/or presentations that result from this study will not identify you by name.

**Participants’ Rights:**

- Your participation in this research study is entirely **voluntary**. If you decide not to participate, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled.

- Your participation or non-participation in this study will in no way affect your grades, your academic standing with CUNY, or any other status in the College.

- Your participation or non-participation in this study will in no way affect your employment at Brooklyn College.

- You can decide to withdraw your consent and stop participating in the research at any time, without any penalty.

**Questions, Comments or Concerns:**
If you have any questions, comments or concerns about the research, you can contact the Principal Investigator, Ashley Polokowski at 718-951-5000 ext. 6651, or at apolokowski@brooklyn.cuny.edu. If you have questions about your rights as a research
participant, or you have comments or concerns that you would like to discuss with someone other than the researchers, please call the CUNY Research Compliance Administrator at 646-664-8918 or email HRPP@cuny.edu. Alternately, you can write to:
CUNY Office of the Vice Chancellor for Research
Attn: Research Compliance Administrator
205 East 42nd Street
New York, NY 10017

**Signature of Participant:**
If you agree to participate in this research study, please sign and date below. You will be given a copy of this consent form to keep.

_____________________________________________________
Printed Name of Participant

_____________________________________________________
Signature of Participant

____________
Date

**Signature of Individual Obtaining Consent**

_____________________________________________________
Printed Name of Individual Obtaining Consent

_____________________________________________________
Signature of Individual Obtaining Consent

____________
Date
Appendix B. Demographic Form

Date (m/d/y):_________ ID#:___________

Baseline Demographic Information

1. What is your age?_________
2. Please specify your gender._____________

3. Please specify your ethnicity.

   White
   Hispanic or Latino
   Black or African American
   Native American or American Indian
   Asian / Pacific Islander
   Other _____________

4. What is the highest degree or level of school you have completed? If currently enrolled, highest degree received.

   No schooling completed
   Nursery school to 8th grade
   Some high school, no diploma
   High school graduate, diploma or the equivalent (for example: GED)
   Some college credit, no degree
   Trade/technical/vocational training
   Associate degree
   Bachelor’s degree
   Master’s degree
   Professional degree
   Doctorate degree

5. What is your marital status?

   Single, never married
   Married or domestic partnership
   Widowed
   Divorced
   Separated

6. Are you currently…?

   Employed for wages
   Self-employed
   Out of work and looking for work
   Out of work but not currently looking for work
A homemaker
A student
Military
Retired
Unable to work

6A. If you are working, how many hours a week do you work? ________

7. What was your total household income during the past 12 months?
   Less than $25,000
   $25,000 to $34,999
   $35,000 to $49,999
   $50,000 to $74,999
   $75,000 to $99,999
   $100,000 to $149,999
   $150,000 or more

8. If you were born in the United States, which region of the country were you born in?
   New England - Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, Vermont
   Middle Atlantic - New Jersey, New York, Pennsylvania
   East North Central - Illinois, Indiana, Michigan, Ohio, Wisconsin
   West North Central - Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, South Dakota
   South Atlantic - Delaware, District of Columbia, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, West Virginia
   East South Central - Alabama, Kentucky, Mississippi, Tennessee
   West South Central - Arkansas, Louisiana, Oklahoma, Texas
   Mountain - Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, Wyoming
   Pacific - Alaska, California, Hawaii, Oregon, Washington

   8A. How long did you live there? __________________

9. If you were not born in the United States, what country were you born in?
   __________________________________________________________________________

   9A. How long did you live there? __________________

10. What neighborhood do you currently live in? __________________________________________

11. How many people currently live in your household? _________________________________

12. Please list any allergies that you have. _____________________________________________

   ______________________________________________________________________________
   ______________________________________________________________________________

13. Please list any dietary restrictions you have.
14. Please note any past or current treatment for anxiety.
Appendix C. Perceived Stress Scale

**PERCEIVED STRESS SCALE**
The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name ____________________________________________________________
Date ______________
Age ________
Gender (Circle): M F Other __________________________

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly? 0 1 2 3 4
2. In the last month, how often have you felt that you were unable to control the important things in your life? 0 1 2 3 4
3. In the last month, how often have you felt nervous and “stressed”? 0 1 2 3 4
4. In the last month, how often have you felt confident about your ability to handle your personal problems? 0 1 2 3 4
5. In the last month, how often have you felt that things were going your way? 0 1 2 3 4
6. In the last month, how often have you found that you could not cope with all the things that you had to do? 0 1 2 3 4
7. In the last month, how often have you been able to control irritations in your life? 0 1 2 3 4
8. In the last month, how often have you felt that you were on top of things? 0 1 2 3 4
9. In the last month, how often have you been angered because of things that were outside of your control? 0 1 2 3 4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them? 0 1 2 3 4
Appendix D. State-Trait Anxiety Inventory

SELF-EVALUATION QUESTIONNAIRE STAI Form Y-1

Please provide the following information:
Name ___________________________ Date ___________ S ______
Age _______________ Gender (Circle) M F T ______

DIRECTIONS:
A number of statements which people have used to describe themselves are given below. Read each statement and then blacken the appropriate circle to the right of the statement to indicate how you feel right now, that is, at this moment. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

1 = Not At All 2 = Somewhat 3 = Moderately So 4 = Very Much So

1. I feel calm................................................................. 1 2 3 4
2. I feel secure................................................................. 1 2 3 4
3. I am tense ................................................................. 1 2 3 4
4. I feel strained ............................................................. 1 2 3 4
5. I feel at ease ............................................................... 1 2 3 4
6. I feel upset..................................................................... 1 2 3 4
7. I am presently worrying over possible misfortunes.............. 1 2 3 4
8. I feel satisfied.................................................................. 1 2 3 4
9. I feel frightened............................................................. 1 2 3 4
10. I feel comfortable......................................................... 1 2 3 4
11. I feel self-confident...................................................... 1 2 3 4
12. I feel nervous ............................................................. 1 2 3 4
13. I am jittery..................................................................... 1 2 3 4
14. I feel indecisive............................................................ 1 2 3 4
15. I am relaxed................................................................. 1 2 3 4
16. I feel content.................................................................. 1 2 3 4
17. I am worried................................................................... 1 2 3 4
18. I feel confused............................................................. 1 2 3 4
19. I feel steady................................................................. 1 2 3 4
20. I feel pleasant.............................................................. 1 2 3 4
SELF-EVALUATION QUESTIONNAIRE STAI Form Y-2

Name ___________________________________________ Date ___________

DIRECTIONS:
A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate you generally feel.

1 = Almost Never = Sometimes 3 = Often 4 = Almost Always

21. I feel pleasant........................................................................................................ 1 2 3 4
22. I feel nervous and restless.................................................................................. 1 2 3 4
23. I feel satisfied with myself.................................................................................. 1 2 3 4
24. I wish I could be as happy as others seem to be ............................................. 1 2 3 4
25. I feel like a failure............................................................................................... 1 2 3 4
26. I feel rested......................................................................................................... 1 2 3 4
27. I am “calm, cool, and collected”......................................................................... 1 2 3 4
28. I feel that difficulties are piling up so that I cannot overcome them............... 1 2 3 4
29. I worry too much over something that really doesn’t matter.......................... 1 2 3 4
30. I am happy........................................................................................................ 1 2 3 4
31. I have disturbing thoughts.................................................................................. 1 2 3 4
32. I lack self-confidence......................................................................................... 1 2 3 4
33. I feel secure........................................................................................................ 1 2 3 4
34. I make decisions easily ...................................................................................... 1 2 3 4
35. I feel inadequate................................................................................................ 1 2 3 4
36. I am content...................................................................................................... 1 2 3 4
37. Some unimportant thought runs through my mind and bothers me............. 1 2 3 4
38. I take disappointments so keenly that I can’t put them out of my mind.......... 1 2 3 4
39. I am a steady person.......................................................................................... 1 2 3 4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests... 1 2 3 4
Appendix E. Beck Depression Inventory

Beck's Depression Inventory
This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1. 0 I do not feel sad.
    1 I feel sad
    2 I am sad all the time and I can't snap out of it.
    3 I am so sad and unhappy that I can't stand it.

2. 0 I am not particularly discouraged about the future.
    1 I feel discouraged about the future.
    2 I feel I have nothing to look forward to.
    3 I feel the future is hopeless and that things cannot improve.

3. 0 I do not feel like a failure.
    1 I feel I have failed more than the average person.
    2 As I look back on my life, all I can see is a lot of failures.
    3 I feel I am a complete failure as a person.

4. 0 I get as much satisfaction out of things as I used to.
    1 I don't enjoy things the way I used to.
    2 I don't get real satisfaction out of anything anymore.
    3 I am dissatisfied or bored with everything.

5. 0 I don't feel particularly guilty
    1 I feel guilty a good part of the time.
    2 I feel quite guilty most of the time.
    3 I feel guilty all of the time.

6. 0 I don't feel I am being punished.
    1 I feel I may be punished.
    2 I expect to be punished.
    3 I feel I am being punished.

7. 0 I don't feel disappointed in myself.
    1 I am disappointed in myself.
    2 I am disgusted with myself.
    3 I hate myself.

8. 0 I don't feel I am any worse than anybody else.
    1 I am critical of myself for my weaknesses or mistakes.
    2 I blame myself all the time for my faults.
    3 I blame myself for everything bad that happens.

9. 0 I don't have any thoughts of killing myself.
    1 I have thoughts of killing myself, but I would not carry them out.
1 I would like to kill myself.
2 I would kill myself if I had the chance.

10. 0 I don't cry any more than usual.
1 I cry more now than I used to.
2 I cry all the time now.
3 I used to be able to cry, but now I can't cry even though I want to.

11. 0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I feel irritated a good deal of the time.
3 I feel irritated all the time.

12. 0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13. 0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14. 0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive.
3 I believe that I look ugly.

15. 0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16. 0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17. 0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18. 0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore.

19. 0 I haven't lost much weight, if any, lately.
1. I have lost more than five pounds.
2. I have lost more than ten pounds.
3. I have lost more than fifteen pounds.

20. 0 I am no more worried about my health than usual.
   1 I am worried about physical problems like aches, pains, upset stomach, or constipation.
   2 I am very worried about physical problems and it's hard to think of much else.
   3 I am so worried about my physical problems that I cannot think of anything else.

21. 0 I have not noticed any recent change in my interest in sex.
   1 I am less interested in sex than I used to be.
   2 I have almost no interest in sex.
   3 I have lost interest in sex completely.
### Appendix X. Skewness, Kurtosis, and Shapiro-Wilk Values for Study Variables

<table>
<thead>
<tr>
<th>Study Variable</th>
<th>Skewness (Std. Error)</th>
<th>Skewness Z-score</th>
<th>Shapiro-Wilk (p-value)</th>
<th>Kurtosis (Std. Error)</th>
<th>Kurtosis Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS</td>
<td>-0.148 (0.271)</td>
<td>0.546</td>
<td>0.982 (0.345)</td>
<td>-0.555 (0.535)</td>
<td>-1.037</td>
</tr>
<tr>
<td>STAI-State</td>
<td>0.565 (0.271)</td>
<td>2.085*</td>
<td>0.961 (0.016)**</td>
<td>-0.238 (0.535)</td>
<td>-0.445</td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>0.065 (0.271)</td>
<td>0.240</td>
<td>0.968 (0.045)**</td>
<td>-1.050 (0.535)</td>
<td>-2.804*</td>
</tr>
<tr>
<td>BDI</td>
<td>0.692 (0.271)</td>
<td>2.553*</td>
<td>0.953 (0.006)**</td>
<td>0.138 (0.535)</td>
<td>0.258</td>
</tr>
<tr>
<td>DHA</td>
<td>0.616 (0.271)</td>
<td>2.273*</td>
<td>0.967 (0.041)**</td>
<td>0.080 (0.535)</td>
<td>0.150</td>
</tr>
<tr>
<td>EPA</td>
<td>1.667 (0.271)</td>
<td>6.151*</td>
<td>0.887 (0.000)**</td>
<td>4.914 (0.535)</td>
<td>9.185*</td>
</tr>
<tr>
<td>ALA</td>
<td>1.172 (0.271)</td>
<td>4.325*</td>
<td>0.919 (0.000)**</td>
<td>1.703 (0.535)</td>
<td>3.183*</td>
</tr>
<tr>
<td>Omega-3 Index</td>
<td>0.849 (0.271)</td>
<td>3.133*</td>
<td>0.952 (0.005)**</td>
<td>1.054 (0.535)</td>
<td>1.970</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5.828 (0.271)</td>
<td>21.506*</td>
<td>0.442 (0.000)**</td>
<td>41.627 (0.535)</td>
<td>77.807*</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.792 (0.271)</td>
<td>17.683*</td>
<td>0.383 (0.000)**</td>
<td>24.087 (0.535)</td>
<td>45.022*</td>
</tr>
<tr>
<td>IL-8</td>
<td>2.895 (0.271)</td>
<td>10.683*</td>
<td>0.696 (0.000)**</td>
<td>10.264 (0.535)</td>
<td>19.185*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>8.597 (0.271)</td>
<td>31.723*</td>
<td>0.151 (0.000)**</td>
<td>75.321 (0.535)</td>
<td>140.787*</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>4.865 (0.293)</td>
<td>16.604*</td>
<td>0.357 (0.000)**</td>
<td>25.707 (0.578)</td>
<td>44.476*</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>2.242 (0.293)</td>
<td>7.652*</td>
<td>0.721 (0.000)**</td>
<td>5.510 (0.578)</td>
<td>9.533*</td>
</tr>
</tbody>
</table>

* Z-score is < -2 or > 2
** Shapiro-Wilk < 0.05
## Appendix Y. Comparison of square root and logarithmic transformation of skewed independent variables

### Square Root Transformation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Skewness (Std. Error)</th>
<th>Skewness Z-score</th>
<th>Shapiro-Wilk (p-value)</th>
<th>Kurtosis (Std. Error)</th>
<th>Kurtosis Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI</td>
<td>-0.085 (0.271)</td>
<td>-0.314</td>
<td>0.984 (0.408)</td>
<td>-0.537 (0.535)</td>
<td>-1.004</td>
</tr>
<tr>
<td>STAI-State</td>
<td>0.277 (0.271)</td>
<td>1.022</td>
<td>0.977 (0.169)</td>
<td>-0.557 (0.535)</td>
<td>-1.041</td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>-0.127 (0.271)</td>
<td>0.469</td>
<td>0.969 (0.054)**</td>
<td>-0.972 (0.535)</td>
<td>-1.817</td>
</tr>
<tr>
<td>DHA</td>
<td>0.303 (0.271)</td>
<td>1.118</td>
<td>0.985 (0.461)</td>
<td>-0.381 (0.535)</td>
<td>-0.712</td>
</tr>
<tr>
<td>EPA</td>
<td>0.813 (0.271)</td>
<td>3.000*</td>
<td>0.962 (0.18)**</td>
<td>1.597 (0.535)</td>
<td>2.985*</td>
</tr>
<tr>
<td>ALA</td>
<td>0.656 (0.271)</td>
<td>2.421*</td>
<td>0.963 (0.023)**</td>
<td>0.816 (0.535)</td>
<td>1.525</td>
</tr>
<tr>
<td>Omega-3 Index</td>
<td>0.561 (0.271)</td>
<td>2.070*</td>
<td>0.972 (0.076)</td>
<td>0.223 (0.535)</td>
<td>0.417</td>
</tr>
<tr>
<td>IL-1β</td>
<td>2.577 (0.271)</td>
<td>9.509*</td>
<td>0.792 (0.000)**</td>
<td>10.788 (0.535)</td>
<td>20.164*</td>
</tr>
<tr>
<td>IL-6</td>
<td>3.301 (0.271)</td>
<td>12.181*</td>
<td>0.648 (0.000)**</td>
<td>12.978 (0.535)</td>
<td>24.258*</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.450 (0.271)</td>
<td>5.351*</td>
<td>0.895 (0.000)**</td>
<td>3.259 (0.535)</td>
<td>6.092*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>6.524 (0.271)</td>
<td>24.074*</td>
<td>0.402 (0.000)**</td>
<td>49.783 (0.535)</td>
<td>93.052*</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>2.748 (0.293)</td>
<td>9.379*</td>
<td>0.673 (0.000)**</td>
<td>8.949 (0.578)</td>
<td>15.483*</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>0.824 (0.293)</td>
<td>2.812*</td>
<td>0.931 (0.001)**</td>
<td>0.261 (0.578)</td>
<td>0.452</td>
</tr>
</tbody>
</table>

* Z-score is <-2 or >2

**Shapiro-Wilk < 0.05

### Logarithmic Transformation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Skewness (Std. Error)</th>
<th>Skewness Z-score</th>
<th>Shapiro-Wilk (p-value)</th>
<th>Kurtosis (Std. Error)</th>
<th>Kurtosis Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI</td>
<td>-1.000 (0.271)</td>
<td>-3.690*</td>
<td>0.918 (0.000)**</td>
<td>0.595 (0.535)</td>
<td>1.112</td>
</tr>
<tr>
<td>STAI-State</td>
<td>-0.007 (0.271)</td>
<td>-0.026</td>
<td>0.982 (0.345)</td>
<td>-0.688 (0.535)</td>
<td>-1.286</td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>-0.337 (0.271)</td>
<td>-1.244</td>
<td>0.964 (0.025)**</td>
<td>-0.753 (0.535)</td>
<td>-1.407</td>
</tr>
<tr>
<td>DHA</td>
<td>0.001 (0.271)</td>
<td>0.004</td>
<td>0.990 (0.795)</td>
<td>-0.548 (0.535)</td>
<td>-1.024</td>
</tr>
<tr>
<td>EPA</td>
<td>0.050 (0.271)</td>
<td>0.185</td>
<td>0.990 (0.788)</td>
<td>0.540 (0.535)</td>
<td>1.009</td>
</tr>
<tr>
<td>ALA</td>
<td>0.069 (0.271)</td>
<td>0.255</td>
<td>0.983 (0.317)</td>
<td>0.773 (0.535)</td>
<td>1.445</td>
</tr>
<tr>
<td>Omega-3 Index</td>
<td>0.306 (0.271)</td>
<td>1.129</td>
<td>0.984 (0.409)</td>
<td>-0.279 (0.535)</td>
<td>-0.521</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>-------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.124</td>
<td>0.458</td>
<td>0.990</td>
<td>0.410</td>
<td>0.766</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.006</td>
<td>3.712*</td>
<td>0.934</td>
<td>2.518</td>
<td>4.707*</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.058</td>
<td>0.214</td>
<td>0.986</td>
<td>0.275</td>
<td>0.514</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.256</td>
<td>4.635*</td>
<td>0.925</td>
<td>4.647</td>
<td>8.686*</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>-0.250</td>
<td>-0.853</td>
<td>0.954</td>
<td>-0.876</td>
<td>-1.521</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>-1.164</td>
<td>-3.973*</td>
<td>0.929</td>
<td>2.172</td>
<td>3.758*</td>
</tr>
</tbody>
</table>

* Z-score is < -2 or > 2
** Shapiro-Wilk < 0.05
### Table 1. Demographic Characteristics (N = 79)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>M ± SD or N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>21.7 ± 4.1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>79 (100.0)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>20 (25.3)</td>
</tr>
<tr>
<td>White</td>
<td>19 (24.1)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>19 (24.1)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>12 (15.2)</td>
</tr>
<tr>
<td>Mixed</td>
<td>6 (7.6)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (3.8)</td>
</tr>
<tr>
<td>Household Income</td>
<td></td>
</tr>
<tr>
<td>Less than $25,000</td>
<td>27 (34.2)</td>
</tr>
<tr>
<td>$25,000-$34,999</td>
<td>9 (11.4)</td>
</tr>
<tr>
<td>$35,000-$49,999</td>
<td>13 (16.5)</td>
</tr>
<tr>
<td>$50,000-$74,999</td>
<td>16 (20.3)</td>
</tr>
<tr>
<td>$75,000-$99,999</td>
<td>4 (5.1)</td>
</tr>
<tr>
<td>$100,000-$149,999</td>
<td>3 (3.8)</td>
</tr>
<tr>
<td>$150,000 or more</td>
<td>7 (8.9)</td>
</tr>
</tbody>
</table>
### Table 2. Descriptive Statistics of Self-Report Measures (N = 79)

<table>
<thead>
<tr>
<th>Measure</th>
<th>M ± SD</th>
<th>Range (Median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSS</td>
<td>20.0 ± 6.6</td>
<td>6.0 - 34.0 (20.0)</td>
</tr>
<tr>
<td>STAI-State</td>
<td>37.4 ± 10.8</td>
<td>20.0 – 67.0 (37.0)</td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>44.9 ± 11.3</td>
<td>23.0 – 68.0 (45.0)</td>
</tr>
<tr>
<td>BDI</td>
<td>12.5 ± 8.0</td>
<td>1.0 – 35.0 (11.0)</td>
</tr>
</tbody>
</table>

*Note.* BDI = Beck Depression Inventory, PSS = Perceived Stress Scale, STAI-State = State-Trait Anxiety Inventory-State, STAI-Trait = State-Trait Anxiety Inventory-Trait.
Table 3. Mean and Standard Deviations for Biological Measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Omega-3 Level</strong></td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>2.69 ± 0.75</td>
</tr>
<tr>
<td>EPA</td>
<td>0.49 ± 0.21</td>
</tr>
<tr>
<td>ALA</td>
<td>0.50 ± 0.17</td>
</tr>
<tr>
<td>ω-3 Index</td>
<td>4.93 ± 1.03</td>
</tr>
<tr>
<td><strong>Inflammatory Markers</strong></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>135.0 ± 238.6</td>
</tr>
<tr>
<td>IL-6</td>
<td>4.7 ± 9.0</td>
</tr>
<tr>
<td>IL-8</td>
<td>725.9 ± 681.0</td>
</tr>
<tr>
<td>TNF-α</td>
<td>7.8 ± 37.9</td>
</tr>
<tr>
<td><strong>Gut Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.81 ± 0.30</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>2.56 ± 0.43</td>
</tr>
</tbody>
</table>

*Note.* For gut bacteria results = (n = 67). ALA = Alpha-linolenic Acid, DHA = Docosahexaenoic Acid, EPA = Eicosapentaenoic Acid, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, TNF-α = Tumor Necrosis Factor Alpha, ω-3- Index = Omega-3 Index.
<table>
<thead>
<tr>
<th></th>
<th>ω-3 Index</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r (p-value)</td>
<td>DHA</td>
<td>EPA</td>
<td>ALA</td>
<td></td>
</tr>
<tr>
<td>PSS</td>
<td>-0.19 (0.09)</td>
<td>-0.21 (0.06)</td>
<td>-0.07 (0.52)</td>
<td>-0.01 (0.92)</td>
<td></td>
</tr>
<tr>
<td>STAI-State</td>
<td>-0.08 (0.47)</td>
<td>-0.09 (0.46)</td>
<td>-0.03 (0.79)</td>
<td>-0.09 (0.42)</td>
<td></td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>-0.29 (0.01)*</td>
<td>-0.30 (0.01)*</td>
<td>-0.18 (0.12)</td>
<td>0.04 (0.71)</td>
<td></td>
</tr>
<tr>
<td>BDI</td>
<td>-0.20 (0.07)</td>
<td>-0.21 (0.06)</td>
<td>-0.13 (0.24)</td>
<td>-0.08 (0.47)</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05

Note. ALA = Alpha-linolenic Acid, BDI = Beck Depression Inventory, DHA = Docosahexaenoic Acid, EPA = Eicosapentaenoic Acid, PSS = Perceived Stress Scale, STAI-State = State-Trait Anxiety Inventory-State, STAI-Trait = State-Trait Anxiety Inventory-Trait, ω-3- Index = Omega-3 Index.
Table 5. Spearman Correlations of ω-3 Levels and Inflammatory Markers (N = 79)

<table>
<thead>
<tr>
<th></th>
<th>ω-3 Index</th>
<th>DHA</th>
<th>EPA</th>
<th>ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>-0.26 (0.02)*</td>
<td>-0.24 (0.03)*</td>
<td>-0.20 (0.08)</td>
<td>-0.05 (0.65)</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.10 (0.39)</td>
<td>-0.14 (0.22)</td>
<td>-0.01 (0.95)</td>
<td>0.06 (0.59)</td>
</tr>
<tr>
<td>IL-8</td>
<td>-0.14 (0.23)</td>
<td>-0.14 (0.21)</td>
<td>-0.11 (0.34)</td>
<td>-0.02 (0.84)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.16 (0.17)</td>
<td>-0.15 (0.18)</td>
<td>-0.11 (0.35)</td>
<td>-0.02 (0.90)</td>
</tr>
</tbody>
</table>

*p<0.05

Note. ALA = Alpha-linolenic Acid, DHA = Docosahexaenoic Acid, EPA = Eicosapentaenoic Acid, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, TNF-α = Tumor Necrosis Factor Alpha, ω-3- Index = Omega-3 Index.
Table 6. Spearman Correlations of ω-3 Levels and Gut Bacteria Abundance (N = 67)

<table>
<thead>
<tr>
<th>Lactobacillus</th>
<th>DHA</th>
<th>EPA</th>
<th>ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.13 (0.29)</td>
<td>-0.09 (0.45)</td>
<td>-0.10 (0.40)</td>
<td>0.06 (0.65)</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>-0.02 (0.89)</td>
<td>-0.01 (0.96)</td>
<td>-0.02 (0.88)</td>
</tr>
</tbody>
</table>

*Note. ALA = Alpha-linolenic Acid, DHA = Docosahexaenoic Acid, EPA = Eicosapentaenoic Acid, ω-3 Index = Omega-3 Index.*
Table 7. Spearman Correlations of Gut Bacteria and Self-report Measures ($N = 67$)

<table>
<thead>
<tr>
<th></th>
<th>Lactobacillus</th>
<th>Bifidobacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$ (p-value)</td>
<td>$r$ (p-value)</td>
<td></td>
</tr>
<tr>
<td>PSS</td>
<td>-0.02 (0.89)</td>
<td>0.11 (0.37)</td>
</tr>
<tr>
<td>STAI-State</td>
<td>-0.05 (0.71)</td>
<td>0.16 (0.20)</td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>-0.01 (0.96)</td>
<td>0.17 (0.16)</td>
</tr>
<tr>
<td>BDI</td>
<td>0.03 (0.84)</td>
<td>0.16 (0.21)</td>
</tr>
</tbody>
</table>

*Note. BDI = Beck Depression Inventory, PSS = Perceived Stress Scale, STAI-State = State-Trait Anxiety Inventory-State, STAI-Trait = State-Trait Anxiety Inventory-Trait.*
Table 8. Spearman Correlations of Inflammatory Markers and Self-report Measures (N = 79)

<table>
<thead>
<tr>
<th></th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-8</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r (p-value)</td>
<td>r (p-value)</td>
<td>r (p-value)</td>
<td>r (p-value)</td>
</tr>
<tr>
<td>PSS</td>
<td>0.21 (0.07)</td>
<td>0.15 (0.19)</td>
<td>0.07 (0.57)</td>
<td>0.05 (0.67)</td>
</tr>
<tr>
<td>STAI-State</td>
<td>0.14 (0.23)</td>
<td>-0.05 (0.67)</td>
<td>-0.05 (0.67)</td>
<td>-0.02 (0.89)</td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>0.17 (0.13)</td>
<td>0.07 (0.55)</td>
<td>0.06 (0.62)</td>
<td>0.03 (0.80)</td>
</tr>
<tr>
<td>BDI</td>
<td>0.26 (0.02)*</td>
<td>0.17 (0.14)</td>
<td>0.14 (0.23)</td>
<td>0.11 (0.34)</td>
</tr>
</tbody>
</table>

*<i>p</i><0.05

Note. BDI = Beck Depression Inventory, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, PSS = Perceived Stress Scale, STAI-State = State-Trait Anxiety Inventory-State, STAI-Trait = State-Trait Anxiety Inventory-Trait, TNF-α = Tumor Necrosis Factor Alpha.
<table>
<thead>
<tr>
<th></th>
<th>Lactobacillus</th>
<th>Bifidobacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-1β</strong></td>
<td>0.11 (0.36)</td>
<td>-0.01 (0.96)</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>-0.05 (0.67)</td>
<td>-0.05 (0.69)</td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td>0.13 (0.31)</td>
<td>0.09 (0.45)</td>
</tr>
<tr>
<td><strong>TNF-α</strong></td>
<td>0.09 (0.50)</td>
<td>-0.04 (0.75)</td>
</tr>
</tbody>
</table>

*Note:* IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, TNF-α = Tumor Necrosis Factor Alpha.
Table 10. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of Omega-3 Index on PSS

<table>
<thead>
<tr>
<th></th>
<th>Lacto → IL-1β</th>
<th>Lacto → IL-8</th>
<th>Lacto → IL-6</th>
<th>Lacto → TNF</th>
<th>Bifido → IL-1β</th>
<th>Bifido → IL-8</th>
<th>Bifido → IL-6</th>
<th>Bifido → TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_1$</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
</tr>
<tr>
<td>$a_2$</td>
<td>-33.75 (29.59)</td>
<td>-74.52 (55.83)</td>
<td>0.11 (0.85)</td>
<td>1.39 (5.03)</td>
<td>-34.39 (29.55)</td>
<td>-71.53 (57.77)</td>
<td>0.13 (0.86)</td>
<td>1.31 (5.03)</td>
</tr>
<tr>
<td>$d$</td>
<td>8.07 (12.30)</td>
<td>57.96 (23.22)*</td>
<td>0.42 (0.35)</td>
<td>-0.13 (2.09)</td>
<td>-6.96 (8.50)</td>
<td>21.45 (16.63)</td>
<td>0.18 (0.25)</td>
<td>-0.72 (1.45)</td>
</tr>
<tr>
<td>$b_1$</td>
<td>0.18 (0.31)</td>
<td>0.14 (0.34)</td>
<td>0.15 (0.32)</td>
<td>0.23 (0.32)</td>
<td>0.18 (0.22)</td>
<td>0.09 (0.23)</td>
<td>0.09 (0.23)</td>
<td>0.11 (0.22)</td>
</tr>
<tr>
<td>$b_2$</td>
<td>0.01 (0.003)†</td>
<td>0.001 (0.002)</td>
<td>0.20 (0.11)†</td>
<td>-0.02 (0.02)</td>
<td>0.01 (0.003)*</td>
<td>0.002 (0.002)</td>
<td>0.20 (0.11)†</td>
<td>-0.02 (0.02)</td>
</tr>
<tr>
<td>$c'$</td>
<td>-1.10 (0.76)</td>
<td>-1.23 (0.79)</td>
<td>-1.37 (0.76)†</td>
<td>-1.32 (0.78)†</td>
<td>-1.06 (0.75)</td>
<td>-1.21 (0.79)</td>
<td>-1.36 (0.79)†</td>
<td>-1.31 (0.79)†</td>
</tr>
<tr>
<td>$c$</td>
<td>-1.34 (0.77)†</td>
<td>-1.34 (0.77)†</td>
<td>-1.34 (0.77)†</td>
<td>-1.34 (0.77)†</td>
<td>-1.34 (0.77)†</td>
<td>-1.34 (0.77)†</td>
<td>-1.34 (0.77)†</td>
<td>-1.34 (0.77)†</td>
</tr>
<tr>
<td>$IE$</td>
<td>0.01 (0.04)</td>
<td>0.001 (0.06)</td>
<td>0.001 (0.07)</td>
<td>0.00 (0.04)</td>
<td>0.01 (0.04)</td>
<td>-0.004 (0.04)</td>
<td>-0.003 (0.04)</td>
<td>-0.001 (0.02)</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.0753, 0.0887</td>
<td>-0.1561, 0.1042</td>
<td>-0.1950, 0.0713</td>
<td>-0.1153, 0.0837</td>
<td>-0.0672, 0.1044</td>
<td>-0.0951, 0.0774</td>
<td>-0.1103, 0.0800</td>
<td>-0.0491, 0.0410</td>
</tr>
</tbody>
</table>

*p<0.10, *p<0.05, **p<0.01

Note. Bifido = Bifidobacterium, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, PSS = Perceived Stress Scale, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
<table>
<thead>
<tr>
<th></th>
<th>Lacto→IL-1β</th>
<th>Lacto→IL-8</th>
<th>Lacto→IL-6</th>
<th>Lacto→TNF</th>
<th>Bifido→IL-1β</th>
<th>Bifido→IL-8</th>
<th>Bifido→IL-6</th>
<th>Bifido→TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>-0.11</td>
<td>-0.11</td>
<td>-0.11</td>
<td>-0.11</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>(0.41)</td>
<td>(0.41)</td>
<td>(0.41)</td>
<td>(0.59)</td>
<td>(0.59)</td>
<td>(0.59)</td>
<td>(0.59)</td>
<td>(0.59)</td>
</tr>
<tr>
<td>a2</td>
<td>-50.87</td>
<td>-116.13</td>
<td>-0.50</td>
<td>-0.02</td>
<td>-50.56</td>
<td>-106.42</td>
<td>-0.43</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>(40.27)</td>
<td>(75.84)</td>
<td>(1.16)</td>
<td>(6.87)</td>
<td>(40.20)</td>
<td>(78.55)</td>
<td>(1.17)</td>
<td>(6.85)</td>
</tr>
<tr>
<td>d</td>
<td>8.59</td>
<td>59.15</td>
<td>0.43</td>
<td>-0.13</td>
<td>-6.91</td>
<td>21.57</td>
<td>0.17</td>
<td>-0.73</td>
</tr>
<tr>
<td></td>
<td>(12.28)</td>
<td>(23.14)**</td>
<td>(0.35)</td>
<td>(2.10)</td>
<td>(8.49)</td>
<td>(16.59)</td>
<td>(0.25)</td>
<td>(1.45)</td>
</tr>
<tr>
<td>b1</td>
<td>0.19</td>
<td>0.17</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.09</td>
<td>0.09</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>(0.31)</td>
<td>(0.34)</td>
<td>(0.32)</td>
<td>(0.22)</td>
<td>(0.22)</td>
<td>(0.23)</td>
<td>(0.22)</td>
<td>(0.22)</td>
</tr>
<tr>
<td>b2</td>
<td>0.01</td>
<td>0.001</td>
<td>0.19</td>
<td>-0.02</td>
<td>0.01</td>
<td>0.002</td>
<td>0.19</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
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<td>(1.05)†</td>
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<td>-0.1494,</td>
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Note. Bifido = Bifidobacterium, DHA = Docosahexaenoic Acid, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, PSS = Perceived Stress Scale, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
Table 12. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of EPA on PSS

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<th>Lacto→IL-6</th>
<th>Lacto→TNF</th>
<th>Bifido→IL-1β</th>
<th>Bifido→IL-8</th>
<th>Bifido→IL-6</th>
<th>Bifido→TNF</th>
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<td>(a1)</td>
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<td>(2.03)</td>
<td>(2.03)</td>
<td>(2.03)</td>
</tr>
<tr>
<td>(a2)</td>
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<td>-62.62</td>
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<td>(275.15)</td>
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<tr>
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<td>(0.12)*</td>
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<td>(0.003)*</td>
<td>(0.002)</td>
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<td>(c')</td>
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<td>-3.03</td>
<td>-5.12</td>
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<tr>
<td>(IE)</td>
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<td>(0.25)</td>
<td>(0.19)</td>
<td>(0.20)</td>
<td>(0.24)</td>
<td>(0.11)</td>
</tr>
<tr>
<td>(95% CIs)</td>
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<td></td>
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<tr>
<td></td>
<td>0.0712, 0.1957, &amp; 0.1676, 0.0697, &amp; 0.4655, 0.3668, &amp; 0.4760, 0.2412,</td>
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\(\dagger<p<0.10, \ast p<0.05, \ast\ast p<0.01\)

**Note.** Bifido = Bifidobacterium, EPA = Eicosapentaenoic Acid, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, PSS = Perceived Stress Scale, TNF-α = Tumor Necrosis Factor Alpha. 95 % CIs = the upper and lower 95 % confidence intervals.
Table 13. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of ALA on PSS

<table>
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<tr>
<th></th>
<th>Lacto→IL-1β</th>
<th>Lacto→IL-8</th>
<th>Lacto→IL-6</th>
<th>Lacto→TNF</th>
<th>Bifido→IL-1β</th>
<th>Bifido→IL-8</th>
<th>Bifido→IL-6</th>
<th>Bifido→TNF</th>
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<td>-1.39</td>
<td>-1.39</td>
<td>-1.39</td>
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<td>0.66</td>
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<tr>
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<td>(1.79)</td>
<td>(2.59)</td>
<td>(2.59)</td>
<td>(2.59)</td>
<td>(2.59)</td>
</tr>
<tr>
<td>a2</td>
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<td>-154.61</td>
<td>0.51</td>
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<td>-248.17</td>
<td>-0.20</td>
<td>-14.55</td>
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<tr>
<td></td>
<td>(179.99)</td>
<td>(341.77)</td>
<td>(5.14)</td>
<td>(30.39)</td>
<td>(178.92)</td>
<td>(350.54)</td>
<td>(5.15)</td>
<td>(30.21)</td>
</tr>
<tr>
<td>d</td>
<td>7.14</td>
<td>56.78</td>
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<td>-6.45</td>
<td>22.46</td>
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<td>(12.43)</td>
<td>(23.61)*</td>
<td>(0.35)</td>
<td>(2.10)</td>
<td>(8.56)</td>
<td>(16.76)</td>
<td>(0.25)</td>
<td>(1.44)</td>
</tr>
<tr>
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<td>0.13</td>
<td>0.17</td>
<td>0.25</td>
<td>0.19</td>
<td>0.08</td>
<td>0.10</td>
<td>0.12</td>
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<tr>
<td></td>
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<td>(0.34)</td>
<td>(0.33)</td>
<td>(0.33)</td>
<td>(0.22)</td>
<td>(0.23)</td>
<td>(0.23)</td>
<td>(0.23)</td>
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<tr>
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<td>0.01</td>
<td>0.002</td>
<td>0.20</td>
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<tr>
<td></td>
<td>(0.003)**</td>
<td>(0.002)</td>
<td>(0.02)†</td>
<td>(0.02)†</td>
<td>(0.003)**</td>
<td>(0.002)</td>
<td>(0.11)†</td>
<td>(0.02)</td>
</tr>
<tr>
<td>c'</td>
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<td>2.48</td>
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<td>3.32</td>
<td>2.67</td>
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<td>1.89</td>
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<td>(4.76)</td>
<td>(4.69)</td>
<td>(4.79)</td>
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<tr>
<td>c</td>
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<td>2.23</td>
<td>2.23</td>
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<td>2.23</td>
<td>2.23</td>
<td>2.23</td>
<td>2.23</td>
</tr>
<tr>
<td>IE</td>
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<td>-0.17</td>
<td>-0.12</td>
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<td>-0.04</td>
<td>0.03</td>
<td>0.02</td>
<td>0.008</td>
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<tr>
<td></td>
<td>(0.25)</td>
<td>(0.28)</td>
<td>(0.32)</td>
<td>(0.22)</td>
<td>(0.16)</td>
<td>(0.19)</td>
<td>(0.19)</td>
<td>(0.09)</td>
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<td>95% CIs</td>
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<td>-0.4381,</td>
<td>-0.6908,</td>
<td>-0.4525,</td>
<td>-0.3045,</td>
<td>-0.3223,</td>
<td>-0.1904,</td>
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<td>0.4398</td>
<td>0.5743</td>
<td>0.1754</td>
<td>0.2580</td>
<td>0.4787</td>
<td>0.4739</td>
<td>0.2027</td>
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</table>

†p<0.10, *p<0.05, **p<0.01

Note. ALA = Alpha-linolenic Acid, Bifido = Bifidobacterium, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, PSS = Perceived Stress Scale, TNF-α = Tumor Necrosis Factor Alpha. 95 % CIs = the upper and lower 95 % confidence intervals.
**Table 14.** Unstandardized Path Coefficients (standard error) for Serial Mediation Model of Omega-3 Index on STAI-Trait

<table>
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<tr>
<th></th>
<th>Lacto® IL-1β</th>
<th>Lacto® IL-8</th>
<th>Lacto® IL-6</th>
<th>Lacto® TNF</th>
<th>Bifido® IL-1β</th>
<th>Bifido® IL-8</th>
<th>Bifido® IL-6</th>
<th>Bifido® TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
</tr>
<tr>
<td>a2</td>
<td>-33.75 (29.59)</td>
<td>-74.52 (55.83)</td>
<td>0.11 (0.85)</td>
<td>1.39 (5.03)</td>
<td>-34.39 (29.55)</td>
<td>-71.53 (57.77)</td>
<td>0.13 (0.86)</td>
<td>1.31 (5.03)</td>
</tr>
<tr>
<td>d</td>
<td>8.07 (12.30)</td>
<td>57.96 (23.22)*</td>
<td>0.42 (0.35)</td>
<td>-0.13 (2.09)</td>
<td>-6.96 (8.50)</td>
<td>21.45 (16.63)</td>
<td>0.18 (0.25)</td>
<td>-0.72 (1.45)</td>
</tr>
<tr>
<td>b1</td>
<td>0.18 (0.56)</td>
<td>0.21 (0.59)</td>
<td>0.25 (0.56)</td>
<td>0.21 (0.56)</td>
<td>0.37 (0.39)</td>
<td>0.34 (0.39)</td>
<td>0.35 (0.39)</td>
<td>0.32 (0.38)</td>
</tr>
<tr>
<td>b2</td>
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<td>-0.001 (0.003)</td>
<td>-0.09 (0.20)</td>
<td>-0.03 (0.03)</td>
<td>0.01 (0.01)</td>
<td>0.002 (0.003)</td>
<td>-0.09 (0.20)</td>
<td>-0.03 (0.03)</td>
</tr>
<tr>
<td>c'</td>
<td>-3.48 (1.35)**</td>
<td>-3.62 (1.36)**</td>
<td>-3.61 (1.34)**</td>
<td>-3.58 (1.34)**</td>
<td>-3.42 (1.35)**</td>
<td>-3.59 (1.36)**</td>
<td>-3.54 (1.33)**</td>
<td></td>
</tr>
<tr>
<td>IE</td>
<td>0.0004 (0.06)</td>
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<td>-0.0004 (0.07)</td>
<td>0.0000 (0.04)</td>
<td>0.0003 (0.03)</td>
<td>0.0004 (0.05)</td>
<td>0.002 (0.04)</td>
<td>-0.002 (0.02)</td>
</tr>
<tr>
<td>95% CIs</td>
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<td>-0.1100, 0.0652</td>
<td>-0.0966, 0.0647</td>
<td>-0.0487, 0.0715</td>
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<td>-0.0673, 0.1186</td>
<td>-0.0497, 0.0492</td>
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</tbody>
</table>

*†p<0.10, *p<0.05, **p<0.01

**Note.** Bifido = Bifidobacterium, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, STAI-Trait = State-Trait Anxiety Inventory-Trait, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
Table 15. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of DHA on STAI-Trait

<table>
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<th></th>
<th>Lacto→IL-1β</th>
<th>Lacto→IL-8</th>
<th>Lacto→IL-6</th>
<th>Lacto→TNF</th>
<th>Bifido→IL-1β</th>
<th>Bifido→IL-8</th>
<th>Bifido→IL-6</th>
<th>Bifido→TNF</th>
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<td>(0.59)</td>
<td>(0.59)</td>
<td>(0.59)</td>
<td>(0.59)</td>
</tr>
<tr>
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<td>-50.56</td>
<td>-106.42</td>
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</tr>
<tr>
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<td>(75.84)</td>
<td>(1.16)</td>
<td>(6.87)</td>
<td>(40.20)</td>
<td>(1.17)</td>
<td>(6.85)</td>
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</tr>
<tr>
<td>d</td>
<td>8.59</td>
<td>59.15</td>
<td>0.43</td>
<td>-6.91</td>
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<td>-0.73</td>
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<td>(12.28)</td>
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<td>(8.49)</td>
<td>(16.59)</td>
<td>(0.25)</td>
<td>(1.45)</td>
<td></td>
</tr>
<tr>
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<td>0.35</td>
<td>0.37</td>
<td>0.32</td>
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<tr>
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<td>(0.59)</td>
<td>(0.57)</td>
<td>(0.39)</td>
<td>(0.39)</td>
<td>(0.39)</td>
<td>(0.38)</td>
<td></td>
</tr>
<tr>
<td>b2</td>
<td>0.004</td>
<td>-0.0003</td>
<td>-0.12</td>
<td>-0.03</td>
<td>-0.0003</td>
<td>-0.12</td>
<td>-0.03</td>
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<tr>
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<td>(0.003)</td>
<td>(0.20)</td>
<td>(0.03)</td>
<td>(0.003)</td>
<td>(0.20)</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>c'</td>
<td>-4.70</td>
<td>-4.93</td>
<td>-4.96</td>
<td>-4.90</td>
<td>-4.60</td>
<td>-4.86</td>
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</tr>
<tr>
<td></td>
<td>(1.85)**</td>
<td>(1.87)**</td>
<td>(1.83)**</td>
<td>(1.82)**</td>
<td>(1.84)*</td>
<td>(1.85)**</td>
<td>(1.82)**</td>
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</tr>
<tr>
<td>c</td>
<td>-4.87</td>
<td>-4.87</td>
<td>-4.87</td>
<td>-4.87</td>
<td>-4.87</td>
<td>-4.87</td>
<td>-4.87</td>
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</tr>
<tr>
<td></td>
<td>(1.81)**</td>
<td>(1.81)**</td>
<td>(1.81)**</td>
<td>(1.81)**</td>
<td>(1.81)**</td>
<td>(1.81)**</td>
<td>(1.81)**</td>
<td></td>
</tr>
<tr>
<td>IE</td>
<td>0.004</td>
<td>-0.002</td>
<td>-0.01</td>
<td>0.003</td>
<td>0.001</td>
<td>0.002</td>
<td>-0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.09)</td>
<td>(0.10)</td>
<td>(0.08)</td>
<td>(0.04)</td>
<td>(0.07)</td>
<td>(0.06)</td>
<td>(0.04)</td>
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<tr>
<td>95% CIs</td>
<td>-0.1330</td>
<td>-0.2405</td>
<td>-0.1760</td>
<td>-0.1385</td>
<td>-0.0733</td>
<td>-0.1514</td>
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<tr>
<td></td>
<td>0.2329</td>
<td>0.2022</td>
<td>0.0959</td>
<td>0.0853</td>
<td>0.0895</td>
<td>0.1399</td>
<td>0.1587</td>
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</tr>
</tbody>
</table>

*p<0.10, *p<0.05, **p<0.01

Note. Bifido = Bifidobacterium, DHA = Docosahexaenoic Acid, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, STAI-Trait = State-Trait Anxiety Inventory-Trait, TNF-α = Tumor Necrosis Factor Alpha. 95 % CIs = the upper and lower 95 % confidence intervals.
Table 16. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of EPA on STAI-Trait

<table>
<thead>
<tr>
<th></th>
<th>Lacto→IL-1β</th>
<th>Lacto→IL-8</th>
<th>Lacto→IL-6</th>
<th>Lacto→TNF</th>
<th>Bifido→IL-1β</th>
<th>Bifido→IL-8</th>
<th>Bifido→IL-6</th>
<th>Bifido→TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>-1.30</td>
<td>-1.30</td>
<td>-1.30</td>
<td>-1.30</td>
<td>-0.81</td>
<td>-0.81</td>
<td>-0.81</td>
<td>-0.81</td>
</tr>
<tr>
<td></td>
<td>(1.40)</td>
<td>(1.40)</td>
<td>(1.40)</td>
<td>(1.40)</td>
<td>(2.03)</td>
<td>(2.03)</td>
<td>(2.03)</td>
<td>(2.03)</td>
</tr>
<tr>
<td>a2</td>
<td>-51.94</td>
<td>-62.62</td>
<td>8.27</td>
<td>28.13</td>
<td>-67.15</td>
<td>-119.19</td>
<td>7.77</td>
<td>27.41</td>
</tr>
<tr>
<td></td>
<td>(141.55)</td>
<td>(268.27)</td>
<td>(3.89)*</td>
<td>(23.61)</td>
<td>(140.49)</td>
<td>(275.15)</td>
<td>(3.92)*</td>
<td>(23.45)</td>
</tr>
<tr>
<td>d</td>
<td>7.48</td>
<td>57.17</td>
<td>0.51</td>
<td>-0.16</td>
<td>-6.86</td>
<td>21.72</td>
<td>0.20</td>
<td>-0.65</td>
</tr>
<tr>
<td></td>
<td>(12.50)</td>
<td>(23.68)*</td>
<td>(0.34)</td>
<td>(2.08)</td>
<td>(8.59)</td>
<td>(16.81)</td>
<td>(0.24)</td>
<td>(1.43)</td>
</tr>
<tr>
<td>b1</td>
<td>0.04</td>
<td>0.02</td>
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<td>0.08</td>
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<td>0.32</td>
<td>0.33</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>(0.58)</td>
<td>(0.60)</td>
<td>(0.59)</td>
<td>(0.58)</td>
<td>(0.39)</td>
<td>(0.40)</td>
<td>(0.40)</td>
<td>(0.40)</td>
</tr>
<tr>
<td>b2</td>
<td>0.01</td>
<td>0.001</td>
<td>0.004</td>
<td>-0.02</td>
<td>0.01</td>
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<td>(0.01)</td>
<td>(0.003)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.01)</td>
<td>(0.003)</td>
<td>(0.01)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>c'</td>
<td>-12.03</td>
<td>-12.25</td>
<td>-12.35</td>
<td>-11.66</td>
<td>-11.74</td>
<td>-12.07</td>
<td>-12.08</td>
<td>-11.56</td>
</tr>
<tr>
<td></td>
<td>(6.51)*</td>
<td>(6.55)*</td>
<td>(6.78)*</td>
<td>(6.60)*</td>
<td>(6.44)*</td>
<td>(6.49)*</td>
<td>(6.68)*</td>
<td>(6.53)*</td>
</tr>
<tr>
<td></td>
<td>(6.41)*</td>
<td>(6.41)*</td>
<td>(6.41)*</td>
<td>(6.41)*</td>
<td>(6.41)*</td>
<td>(6.41)*</td>
<td>(6.41)*</td>
<td>(6.41)*</td>
</tr>
<tr>
<td>IE</td>
<td>-0.05</td>
<td>-0.08</td>
<td>-0.005</td>
<td>0.005</td>
<td>0.03</td>
<td>-0.01</td>
<td>0.001</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>(0.26)</td>
<td>(0.34)</td>
<td>(0.30)</td>
<td>(0.22)</td>
<td>(0.17)</td>
<td>(0.22)</td>
<td>(0.20)</td>
<td>(0.11)</td>
</tr>
<tr>
<td>95%</td>
<td>-0.8949,</td>
<td>-0.9434,</td>
<td>-0.7019,</td>
<td>-0.6500,</td>
<td>-0.2867,</td>
<td>-0.4555,</td>
<td>-0.3163,</td>
<td>-0.1639,</td>
</tr>
<tr>
<td>CIs</td>
<td>0.0561,</td>
<td>0.5371</td>
<td>0.3789</td>
<td>0.2444</td>
<td>0.3771</td>
<td>0.3986</td>
<td>0.4280</td>
<td>0.2219</td>
</tr>
</tbody>
</table>

†p<0.10, *p<0.05, **p<0.01

Note. Bifido = Bifidobacterium, EPA = Eicosapentaenoic Acid, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, STAI-Trait = State-Trait Anxiety Inventory-Trait, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
Table 17. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of ALA on STAI-Trait

<table>
<thead>
<tr>
<th></th>
<th>Lacto → IL-1β</th>
<th>Lacto → IL-8</th>
<th>Lacto → IL-6</th>
<th>Lacto → TNF</th>
<th>Bifido → IL-1β</th>
<th>Bifido → IL-8</th>
<th>Bifido → IL-6</th>
<th>Bifido → TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>-1.39 (1.79)</td>
<td>-1.39 (1.79)</td>
<td>-1.39 (1.79)</td>
<td>-1.39 (1.79)</td>
<td>0.66 (2.59)</td>
<td>0.66 (2.59)</td>
<td>0.66 (2.59)</td>
<td>0.66 (2.59)</td>
</tr>
<tr>
<td>a2</td>
<td>-131.10 (179.99)</td>
<td>-154.61 (341.77)</td>
<td>0.51 (5.14)</td>
<td>-15.33 (30.39)</td>
<td>-136.73 (178.92)</td>
<td>-248.17 (350.54)</td>
<td>-0.20 (5.15)</td>
<td>-14.55 (30.21)</td>
</tr>
<tr>
<td>d</td>
<td>7.14 (12.43)</td>
<td>56.78 (23.61)*</td>
<td>0.43 (0.35)</td>
<td>-0.23 (2.10)</td>
<td>-6.45 (8.56)</td>
<td>22.46 (16.76)</td>
<td>0.18 (0.25)</td>
<td>-0.71 (1.44)</td>
</tr>
<tr>
<td>b1</td>
<td>0.17 (0.59)</td>
<td>0.15 (0.62)</td>
<td>0.26 (0.60)</td>
<td>0.21 (0.59)</td>
<td>0.41 (0.40)</td>
<td>0.34 (0.41)</td>
<td>0.38 (0.41)</td>
<td>0.34 (0.41)</td>
</tr>
<tr>
<td>b2</td>
<td>0.01 (0.01)</td>
<td>0.001 (0.003)</td>
<td>-0.09 (0.21)</td>
<td>-0.03 (0.04)</td>
<td>0.01 (0.01)</td>
<td>0.001 (0.003)</td>
<td>-0.10 (0.21)</td>
<td>-0.03 (0.04)</td>
</tr>
<tr>
<td>c'</td>
<td>3.20 (8.54)</td>
<td>2.57 (8.59)</td>
<td>2.42 (8.57)</td>
<td>1.89 (8.55)</td>
<td>2.79 (8.45)</td>
<td>2.10 (8.53)</td>
<td>1.81 (8.49)</td>
<td>1.39 (8.47)</td>
</tr>
<tr>
<td>c</td>
<td>2.07 (8.42)</td>
<td>2.07 (8.42)</td>
<td>2.07 (8.42)</td>
<td>2.07 (8.42)</td>
<td>2.07 (8.42)</td>
<td>2.07 (8.42)</td>
<td>2.07 (8.42)</td>
<td>2.07 (8.42)</td>
</tr>
<tr>
<td>IE</td>
<td>-0.06 (0.39)</td>
<td>-0.10 (0.38)</td>
<td>0.06 (0.35)</td>
<td>-0.01 (0.25)</td>
<td>-0.03 (0.16)</td>
<td>0.02 (0.20)</td>
<td>-0.01 (0.17)</td>
<td>0.01 (0.11)</td>
</tr>
<tr>
<td>95% CIs</td>
<td>-1.3148, 0.1945</td>
<td>-0.7724, 0.7518</td>
<td>-0.2739, 0.5357</td>
<td>-0.5683, 0.3897</td>
<td>-0.4001, 0.2445</td>
<td>-0.4469, 0.3690</td>
<td>-0.4754, 0.1745</td>
<td>-0.2498, 0.2221</td>
</tr>
</tbody>
</table>

Note. ALA = Alpha-linolenic Acid, Bifido = Bifidobacterium, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, STAI-Trait = State-Trait Anxiety Inventory-Trait, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.

†p<0.10, *p<0.05, **p<0.01
### Table 18. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of Omega-3 Index on BDI

<table>
<thead>
<tr>
<th></th>
<th>Lacto→IL-1β</th>
<th>Lacto→IL-8</th>
<th>Lacto→IL-6</th>
<th>Lacto→TNF</th>
<th>Bifido→IL-1β</th>
<th>Bifido→IL-8</th>
<th>Bifido→IL-6</th>
<th>Bifido→TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>0.01 (0.30)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
<td>-0.11 (0.43)</td>
<td></td>
</tr>
<tr>
<td>a2</td>
<td>-33.75 (29.59)</td>
<td>-74.52 (55.83)</td>
<td>0.11 (0.85)</td>
<td>1.39 (5.03)</td>
<td>-34.39 (29.55)</td>
<td>-71.53 (57.77)</td>
<td>0.13 (0.86)</td>
<td>1.31 (5.03)</td>
</tr>
<tr>
<td>d</td>
<td>8.07 (12.30)</td>
<td>57.96 (23.22)*</td>
<td>0.42 (0.35)</td>
<td>-0.13 (2.09)</td>
<td>-6.96 (8.50)</td>
<td>21.45 (16.63)</td>
<td>0.18 (0.25)</td>
<td>-0.72 (1.45)</td>
</tr>
<tr>
<td>b1</td>
<td>0.53 (0.36)</td>
<td>0.50 (0.38)</td>
<td>0.56 (0.37)</td>
<td>0.57 (0.37)</td>
<td>0.29 (0.25)</td>
<td>0.21 (0.26)</td>
<td>0.25 (0.26)</td>
<td>0.24 (0.26)</td>
</tr>
<tr>
<td>b2</td>
<td>0.004 (0.004)</td>
<td>0.001 (0.002)</td>
<td>0.01 (0.13)</td>
<td>-0.02 (0.02)</td>
<td>0.01 (0.004)</td>
<td>0.002 (0.002)</td>
<td>0.03 (0.13)</td>
<td>-0.02 (0.02)</td>
</tr>
<tr>
<td>c'</td>
<td>-1.72 (0.88)†</td>
<td>-1.79 (0.90)*</td>
<td>-1.88 (0.89)*</td>
<td>-1.85 (0.89)*</td>
<td>-1.65 (0.89)†</td>
<td>-1.72 (0.90)†</td>
<td>-1.84 (0.90)*</td>
<td>-1.82 (0.89)*</td>
</tr>
<tr>
<td>c</td>
<td>-1.87 (0.89)*</td>
<td>-1.87 (0.89)*</td>
<td>-1.87 (0.89)*</td>
<td>-1.87 (0.89)*</td>
<td>-1.87 (0.89)*</td>
<td>-1.87 (0.89)*</td>
<td>-1.87 (0.89)*</td>
<td>-1.87 (0.89)*</td>
</tr>
<tr>
<td>IE</td>
<td>0.0005 (0.04)</td>
<td>0.0008 (0.05)</td>
<td>0.0000 (0.05)</td>
<td>0.0000 (0.03)</td>
<td>0.004 (0.03)</td>
<td>-0.004 (0.04)</td>
<td>-0.0005 (0.03)</td>
<td>-0.002 (0.02)</td>
</tr>
<tr>
<td>95%</td>
<td>-0.0702,-0.1335,</td>
<td>-0.1192,-0.0859,</td>
<td>0.0451,-0.0541,</td>
<td>0.0501,-0.0737,</td>
<td>-0.1053,-0.0573,</td>
<td>-0.0572,-0.0439,</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†p<0.10, *p<0.05, **p<0.01

Note. BDI = Beck Depression Inventory, Bifido = Bifidobacterium, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
Table 19. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of DHA on BDI

<table>
<thead>
<tr>
<th></th>
<th>Lacto → IL-1β</th>
<th>Lacto → IL-8</th>
<th>Lacto → IL-6</th>
<th>Lacto → TNF</th>
<th>Bifido → IL-1β</th>
<th>Bifido → IL-8</th>
<th>Bifido → IL-6</th>
<th>Bifido → TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>0.12 (0.41)</td>
<td>0.12 (0.41)</td>
<td>0.12 (0.41)</td>
<td>0.12 (0.41)</td>
<td>-0.11 (0.59)</td>
<td>-0.11 (0.59)</td>
<td>-0.11 (0.59)</td>
<td>-0.11 (0.59)</td>
</tr>
<tr>
<td>a2</td>
<td>-50.87 (40.27)</td>
<td>-116.13 (75.84)</td>
<td>-0.50 (1.16)</td>
<td>-0.02 (6.87)</td>
<td>-50.56 (40.20)</td>
<td>-106.42 (78.55)</td>
<td>-0.43 (1.17)</td>
<td>0.12 (6.85)</td>
</tr>
<tr>
<td>d</td>
<td>8.59 (12.38)</td>
<td>59.15 (23.14)**</td>
<td>0.43 (0.35)</td>
<td>-0.13 (2.10)</td>
<td>-6.91 (8.49)</td>
<td>21.57 (16.59)</td>
<td>0.17 (0.25)</td>
<td>-0.73 (1.45)</td>
</tr>
<tr>
<td>b1</td>
<td>0.55 (0.37)</td>
<td>0.53 (0.39)</td>
<td>0.60 (0.37)</td>
<td>0.59 (0.37)</td>
<td>0.29 (0.26)</td>
<td>0.22 (0.26)</td>
<td>0.25 (0.26)</td>
<td>0.24 (0.26)</td>
</tr>
<tr>
<td>b2</td>
<td>0.005 (0.004)</td>
<td>-0.001 (0.002)</td>
<td>-0.01 (0.13)</td>
<td>-0.02 (0.02)</td>
<td>0.01 (0.004)</td>
<td>0.002 (0.002)</td>
<td>0.01 (0.13)</td>
<td>-0.02 (0.02)</td>
</tr>
<tr>
<td>c'</td>
<td>-2.23 (1.21)†</td>
<td>-2.34 (1.23)†</td>
<td>-2.47 (1.21)*</td>
<td>-2.47 (1.20)*</td>
<td>-2.09 (1.22)†</td>
<td>-2.19 (1.24)†</td>
<td>-2.36 (1.23)†</td>
<td>-2.37 (1.22)†</td>
</tr>
<tr>
<td>c</td>
<td>-2.40 (1.22)*</td>
<td>-2.40 (1.22)*</td>
<td>-2.40 (1.22)*</td>
<td>-2.40 (1.22)*</td>
<td>-2.40 (1.22)*</td>
<td>-2.40 (1.22)*</td>
<td>-2.40 (1.22)*</td>
<td>-2.40 (1.22)*</td>
</tr>
<tr>
<td>IE</td>
<td>0.005 (0.07)</td>
<td>0.008 (0.08)</td>
<td>-0.0005 (0.07)</td>
<td>0.0003 (0.05)</td>
<td>0.004 (0.04)</td>
<td>-0.004 (0.05)</td>
<td>-0.0002 (0.03)</td>
<td>-0.002 (0.03)</td>
</tr>
<tr>
<td>95% CIs</td>
<td>-0.0961, 0.1646</td>
<td>-0.1884, 0.1363</td>
<td>-0.1438, 0.0633</td>
<td>-0.1294, 0.0871</td>
<td>-0.0745, 0.0922</td>
<td>-0.1338, 0.0768</td>
<td>-0.0711, 0.0665</td>
<td>-0.0649, 0.0478</td>
</tr>
</tbody>
</table>

†p<0.10, *p<0.05, **p<0.01

Note. BDI = Beck Depression Inventory, Bifido = Bifidobacterium, DHA = Docosahexaenoic Acid, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
Table 20. Unstandardized Path Coefficients (standard error) for Serial Mediation Model of EPA on BDI

<table>
<thead>
<tr>
<th></th>
<th>Lacto→ IL-1β</th>
<th>Lacto→ IL-8</th>
<th>Lacto→ IL-6</th>
<th>Lacto→ TNF</th>
<th>Bifido→ IL-1β</th>
<th>Bifido→ IL-8</th>
<th>Bifido→ IL-6</th>
<th>Bifido→ TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
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<td>(1.40)</td>
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<td>(2.03)</td>
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<td>a2</td>
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<td>8.27</td>
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<td>-67.15</td>
<td>-119.19</td>
<td>7.77</td>
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<td>(141.55)</td>
<td>(268.27)</td>
<td>(3.89)*</td>
<td>(23.61)</td>
<td>(140.49)</td>
<td>(275.15)</td>
<td>(3.92)*</td>
<td>(23.45)</td>
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<td>(12.50)</td>
<td>(23.68)*</td>
<td>(0.34)</td>
<td>(2.08)</td>
<td>(8.59)</td>
<td>(16.81)</td>
<td>(0.24)</td>
<td>(1.43)</td>
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<tr>
<td>b1</td>
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<td>0.39</td>
<td>0.46</td>
<td>0.50</td>
<td>0.29</td>
<td>0.20</td>
<td>0.23</td>
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<tr>
<td></td>
<td>(0.37)</td>
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<td>(0.38)</td>
<td>(0.37)</td>
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<tr>
<td>b2</td>
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<td>0.002</td>
<td>0.07</td>
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<td>0.01</td>
<td>0.002</td>
<td>0.08</td>
<td>-0.02</td>
</tr>
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<td></td>
<td>(0.004)</td>
<td>(0.002)</td>
<td>(0.02)</td>
<td>(0.02)</td>
<td>(0.004)</td>
<td>(0.002)</td>
<td>(0.14)</td>
<td>(0.02)</td>
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<tr>
<td>c'</td>
<td>-6.91</td>
<td>-7.08</td>
<td>-7.74</td>
<td>-6.72</td>
<td>-7.21</td>
<td>-7.38</td>
<td>-8.28</td>
<td>-7.22</td>
</tr>
<tr>
<td></td>
<td>(4.18)†</td>
<td>(4.23)†</td>
<td>(4.39)†</td>
<td>(4.28)</td>
<td>(4.17)†</td>
<td>(4.22)†</td>
<td>(4.37)†</td>
<td>(4.28)†</td>
</tr>
<tr>
<td></td>
<td>(4.21)†</td>
<td>(4.21)†</td>
<td>(4.21)†</td>
<td>(4.21)†</td>
<td>(4.21)†</td>
<td>(4.21)†</td>
<td>(4.21)†</td>
<td>(4.21)†</td>
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<tr>
<td>IE</td>
<td>-0.05</td>
<td>-0.04</td>
<td>-0.04</td>
<td>0.003</td>
<td>0.03</td>
<td>-0.04</td>
<td>-0.01</td>
<td>-0.008</td>
</tr>
<tr>
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<td>(0.16)</td>
<td>(0.23)</td>
<td>(0.19)</td>
<td>(0.15)</td>
<td>(0.15)</td>
<td>(0.17)</td>
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<tr>
<td>95% CI</td>
<td>-0.5315, 0.0070</td>
<td>-0.7117, 0.1232</td>
<td>-0.6706, 0.1930</td>
<td>-0.5753, 0.3632</td>
<td>-0.2524, 0.3073</td>
<td>-0.3998, 0.3073</td>
<td>-0.3492, 0.2817</td>
<td>-0.1687, 0.2075</td>
</tr>
</tbody>
</table>

†p<0.10, *p<0.05, **p<0.01

Note. BDI = Beck Depression Inventory, Bifido = Bifidobacterium, EPA = Eicosapentaeonoic Acid, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
**Table 21.** Unstandardized Path Coefficients (standard error) for Serial Mediation Model of ALA on BDI

<table>
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<tr>
<th></th>
<th>Lacto→ IL-1β</th>
<th>Lacto→ IL-6</th>
<th>Lacto→ IL-8</th>
<th>Lacto→ TNF</th>
<th>Bifido→ IL-1β</th>
<th>Bifido→ IL-6</th>
<th>Bifido→ IL-8</th>
<th>Bifido→ TNF</th>
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<tr>
<td>(a1)</td>
<td>-1.39 (1.79)</td>
<td>-1.39 (1.79)</td>
<td>-1.39 (1.79)</td>
<td>-1.39 (1.79)</td>
<td>0.66 (2.59)</td>
<td>0.66 (2.59)</td>
<td>0.66 (2.59)</td>
<td>0.66 (2.59)</td>
</tr>
<tr>
<td>(a2)</td>
<td>-131.10 (179.99)</td>
<td>-154.61 (341.77)</td>
<td>0.51 (5.14)</td>
<td>-15.33 (30.39)</td>
<td>-136.73 (178.92)</td>
<td>-248.17 (350.54)</td>
<td>-0.20 (5.15)</td>
<td>-14.55 (30.21)</td>
</tr>
<tr>
<td>(d)</td>
<td>7.14 (12.43)</td>
<td>56.78 (23.61)*</td>
<td>0.43 (0.35)</td>
<td>-0.23 (2.10)</td>
<td>-6.45 (8.56)</td>
<td>-22.46 (16.76)</td>
<td>0.18 (0.25)</td>
<td>-0.71 (1.44)</td>
</tr>
<tr>
<td>(b1)</td>
<td>0.52 (0.38)</td>
<td>0.46 (0.40)</td>
<td>0.56 (0.39)</td>
<td>0.55 (0.38)</td>
<td>0.31 (0.26)</td>
<td>0.22 (0.27)</td>
<td>0.27 (0.27)</td>
<td>0.26 (0.26)</td>
</tr>
<tr>
<td>(b2)</td>
<td>0.01 (0.004)</td>
<td>0.002 (0.002)</td>
<td>0.01 (0.13)</td>
<td>-0.02 (0.02)</td>
<td>0.01 (0.004)†</td>
<td>0.002 (0.002)</td>
<td>0.02 (0.14)</td>
<td>-0.02 (0.03)</td>
</tr>
<tr>
<td>(c')</td>
<td>-0.01 (5.47)</td>
<td>-0.48 (5.51)</td>
<td>-0.76 (5.54)</td>
<td>-1.10 (5.51)</td>
<td>-0.83 (5.47)</td>
<td>-1.16 (5.53)</td>
<td>-1.71 (5.57)</td>
<td>-2.02 (5.54)</td>
</tr>
<tr>
<td>(c)</td>
<td>-1.54 (5.52)</td>
<td>-1.54 (5.52)</td>
<td>-1.54 (5.52)</td>
<td>-1.54 (5.52)</td>
<td>-1.54 (5.52)</td>
<td>-1.54 (5.52)</td>
<td>-1.54 (5.52)</td>
<td>-1.54 (5.52)</td>
</tr>
<tr>
<td>(IE)</td>
<td>-0.06 (0.26)</td>
<td>-0.14 (0.24)</td>
<td>-0.003 (0.23)</td>
<td>-0.007 (0.18)</td>
<td>-0.03 (0.14)</td>
<td>0.03 (0.16)</td>
<td>0.003 (0.13)</td>
<td>0.001 (0.01)</td>
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<tr>
<td>95% CIs</td>
<td>-0.8366, 0.1427</td>
<td>-0.5228, 0.4035</td>
<td>-0.2807, 0.3106</td>
<td>-0.4547, 0.2656</td>
<td>-0.3305, 0.2348</td>
<td>-0.3305, 0.3674</td>
<td>-0.2353, 0.2186</td>
<td>-0.1983, 0.1826</td>
</tr>
</tbody>
</table>

†\(p<0.10, *p<0.05, **p<0.01\)

Note. ALA = Alpha-linolenic Acid, BDI = Beck Depression Inventory, Bifido = Bifidobacterium, IE = Indirect Effect of the serial mediation, IL-1β = Interleukin-1 Beta, IL-6 = Interleukin-6, IL-8 = Interleukin-8, Lacto = Lactobacillus, TNF-α = Tumor Necrosis Factor Alpha. 95% CIs = the upper and lower 95% confidence intervals.
Figure 1. Inflammation as a hypothesized mechanism of action explaining the relationship between ω-3 FAs and anxiety and depression.
Figure 2. Proposed path analysis examining inflammation and gut bacteria as a hypothesized mechanism of action explaining the relationship between ω-3 FAs and stress symptoms.
Figure 3. Proposed path analysis examining inflammation and gut bacteria as a hypothesized mechanism of action explaining the relationship between ω-3 FAs and anxiety symptoms.
Figure 4. Proposed path analysis examining inflammation and gut bacteria as a hypothesized mechanism of action explaining the relationship between ω-3 FAs and depression symptoms.
Figure 5. Proposed serial mediation model, key for mediation results in Aim 2.