SUPPLEMENTATION OF OMEGA-3 FATTY ACIDS AND PROBIOTICS: EMERGING NUTRITIONAL INTERVENTIONS FOR MAJOR DEPRESSIVE DISORDER

by
MELISSA GORECKI, RD, CD

Submitted in partial fulfillment of the requirements
for the degree
Master of Science in Dietetics

MOUNT MARY UNIVERSITY

December, 2018

Approved by:

Megan Baumler, PhD, RD
Professor, Graduate Program in Dietetics

Tara L. LaRowe, PhD, RD, CD
Professor, Graduate Program in Dietetics

SUPPLEMENTATION OF OMEGA-3 FATTY ACIDS AND PROBIOTICS: EMERGING NUTRITIONAL INTERVENTIONS FOR MAJOR DEPRESSIVE DISORDER

Melissa Gorecki, RD, CD
December 14th, 2018

ABSTRACT OF THESIS

OBJECTIVE: To critically analyze the current research on probiotic and omega-3 fatty acid supplementation as means to reduce depressive symptom severity in adults with Major Depressive Disorder (MDD).

DESIGN: Academy of Nutrition and Dietetics (AND) Evidence Analysis Library (EAL) Project.

METHODS: An EAL Project was conducted using the systematic process set forth by AND. A total of five methodical steps were undertaken: 1) Formulate the Evidence Analysis Question, 2) Gather and Classify the Evidence, 3) Critically Appraise Each Article, 4) Summarize the Evidence, and 5) Write and Grade the Conclusion Statement.

RESULTS: A literature search resulted in >50 hits with 19 articles selected for further review. Nine articles were excluded due to not meeting the defined inclusion/exclusion criteria. A total of five articles were selected for review for each of the two research questions posed. All 10 studies were randomized controlled trials. Study populations ranged in size from 40-432 participants. All study participants either met criteria for MDD as defined by DSM-IV, or were confirmed to have depressive symptoms at screening using a validated tool. Participants ranged in age from 18-73. All studies involved the provision of either an omega-3 fatty acid or probiotic supplement for treatment of depression symptoms. The primary outcome of each study was depressive symptom severity as measured by a validated tool. The Hamilton Depression Rating Scale (HDRS, HAM-D) was utilized as the primary outcome measure in 7 of the 10 studies analyzed. Study quality was evaluated using the Quality Criteria Checklist published by AND. All ten studies were rated positive (+) for study quality and design.

CONCLUSION: The current research does not indicate that omega-3 or probiotic supplementation is more effective as a monotherapy for MDD than conventional treatment or placebo. A number of studies suggest the utility of providing an omega-3 or probiotic supplement in conjunction with a selective serotonin reuptake inhibitor (SSRI) or tricyclic antidepressant to provide a greater reduction in depressive symptoms compared to conventional treatment. This conclusion was given the grade *Fair, II.*

ACKNOWLEDGMENTS

I would like to thank all of the exceptional faculty of the Mount Mary University Coordinated Program in Dietetics and Graduate Program in Dietetics that I have had the honor of working with during the past 8 years. I am forever grateful for the knowledge, patience, and generosity that you have shown me. In particular, I would like to thank Dr. Megan Baumler for the deep level of dedication and enthusiasm she has shown as my advisor and mentor during my journey as a graduate student. Although words cannot fully express my love and gratitude, I would like to thank my Mom for being my rock and biggest cheerleader in all aspects of my life. I love you more! Finally, I would like to thank my husband, Chris, for being the inspiration behind this research project. Your commitment to your health and your journey to the other side of depression has been so inspiring to me. I am so proud of you and love you very much.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER1: INTRODUCTION

Clinical depression is a general term that encapsulates several disorders with specific diagnostic criteria. According to the National Institute of Mental Health, the specific condition known as major depressive disorder (MDD) can be defined as "a period of two weeks or longer during which there is either depressed mood or anhedonia, and at least four other symptoms that reflect a change in functioning" (24). These symptoms include hopelessness, irritability, difficulty concentrating, social withdrawal, sleep disturbances, change in appetite, musculoskeletal pain, gastrointestinal distress, and low libido (4, 6).

It has been estimated that 300 million individuals are affected by depression worldwide; while close to 7% of the adult population in the United States suffer from the disorder (5, 24). The implications of depression are far-reaching. Due to its host of associated symptoms, depression may significantly impact an individual's quality of life and ability to adequately perform in social, academic, and professional settings. Incidence of depression has also been shown to be associated with a variety of chronic diseases such as metabolic syndrome, cardiovascular disease, diabetes mellitus, cancer, and Parkinson's disease (4, 30). Given this, depression is among the top five causes of disability globally (3). When symptoms are severe or untreated, risk of suicide increases significantly. According to the World Health Organization, approximately 800, 000 people die as a result of suicide every year (5).

While current treatment options for depression are varied, the gold standard of care remains pharmacotherapy combined with psychotherapy (4). Intractable cases may require more aggressive measures such as inpatient hospitalization or electroconvulsive

therapy (ECT). Despite widespread use, most depression medications are associated with a number of side effects that may negatively impact quality of life and treatment compliance. For instance, side effects of the medication class known as selective serotonin reuptake inhibitors (SSRIs) include weight gain, sleep disturbances, sexual dysfunction, nausea, headaches, anxiety, and agitation (7). Further, the efficacy of both individual and combined treatment modalities for depression have been called into question (17). Research has suggested that standard combined treatment fails to achieve remission in greater than 30% of depressed individuals (25). Findings such as this, coupled with the risk of significant medication side effects, may explain the recent interest in alternative interventions to address depression. Interestingly, data from the 2012 National Health Interview Survey suggests that 38% of adults in the United States have used some form of a "complementary health approach" (20). Other research has indicated that use of complementary and alternative medicine (CAM) may be even more prevalent in individuals with depression and anxiety. A survey conducted two decades ago found that approximately 67% of respondents with severe depression pursued "complementary and alternative therapies" (13).

Given the relatively low efficacy of conventional treatment and the growing interest in CAM and integrative medicine, a significant amount of interest has focused on the relationship of nutrition and mental health. Despite this, current medical nutrition therapy for depression is still essentially limited to the promotion of a nutritionally balanced diet in order to minimize the risk of nutritional deficiencies that may exacerbate depressive symptoms. While this is undoubtedly a critical component of managing depression, this intervention does not specifically target the proposed mechanisms for the development and perpetuation of depression.

A relatively complex risk factor set has been identified for depression. Genetic predisposition, altered neurological function, environmental triggers, and traumatic or stressful life events are some of the most common risk factors for the disorder (5, 15). Current treatment options for depression are typically aimed at the pathophysiology associated with these factors. However, an emerging body of research has implicated additional pathways for the development of depression. In particular, recent research has focused on the associations between depression and systemic inflammation, oxidative stress and gut-brain interactions (20). As the mechanisms of these associations are uncovered, a number of targeted treatment options have been proposed, many of which are nutrition-based.

Undoubtedly, it is of utmost importance that the large body of research related to the potential nutritional interventions for depression be critically analyzed and translated into updated, evidence-based guidelines for dietetics practitioners. By utilizing a rigorous and objective process such as the Evidence Analysis Process designed by the Academy of Nutrition and Dietetics, the final results and recommendations of such a project could have the potential to provide evidentiary support for expanding the medical nutrition therapy guidelines for the treatment of depression. In theory, this could equip the Registered Dietitian Nutritionist (RDN) with the resources to implement highly individualized, evidence-based nutritional interventions that target the unique etiologies of depression. Ultimately, RDNs may be able to play a significantly enhanced role in improving quality of life and reducing disease burden in those individuals with MDD.

Research Question

This investigator proposes to perform a systematic evidence-analysis project to critically analyze research specifically related to nutritional interventions designed to

reduce depressive symptoms by way of modulating systemic inflammation and oxidative stress. This investigator's formal research questions are as follows:

1) In adults with Major Depressive Disorder (MDD), how effective is polyunsaturated omega-3 fatty acid supplementation at reducing depressive symptom severity compared to conventional treatment or placebo?
2) In adults with MDD, how effective is probiotic supplementation at reducing depressive symptom severity compared to conventional treatment or placebo?

Limitations

Since these research questions have been proposed with an evidence analysis project in mind, the main limitation of this research project will be the volume and quality of existing research. In particular, it is anticipated that many studies available for consideration may have relevant interventions and outcomes but not a relevant study population, or vice versa. Another likely limitation is the constraints imposed by the actual evidence-analysis process, as it requires adherence to a rigorously defined methodology.

Delimitations

While the volume of existing relevant research is the core limitation to this research project, the specific population, interventions, dependent variables, and other inclusion and exclusion criteria that will be intentionally imposed by this investigator will be the main delimitations of the research project. It is anticipated that modifications to the inclusion and exclusion criteria will be inevitable in order to yield an appropriate volume of research for evaluation.

Assumptions

Since this investigator will be reliant on the evidence analysis process defined by the Academy of Nutrition and Dietetics, the primary assumption of this research project will be that the Academy's methodology is ultimately a reliable, rigorous, and objective process to compile and critically analyze nutrition research. Additionally, it will be assumed that the published research selected for this project is both truthful and accurate.

Definitions

Anhedonia: a decreased ability to feel pleasure. The two main categories of anhedonia are social and physical anhedonia (32).

Bacterial translocation: the passage of bacteria from the gastrointestinal tract to extraintestinal locations, such as the bloodstream or lymphatic system, by way of increased permeability of the intestinal mucosa, immune dysfunction, or intestinal bacterial overgrowth (2).

Cytokines: a general term for small proteins secreted by cells that influence intercellular interaction and communication. Cytokines may be pro-inflammatory or anti-inflammatory. They are often produced in a cascade and their action may be synergistic or antagonistic. Three examples of major pro-inflammatory cytokines are interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) (40).

Glutathione: a major antioxidant in all mammalian tissues. It is a non-protein thiol responsible for modulating oxidative stress as well as a vast number of other cellular processes such as apoptosis, cell proliferation, fibrogenesis, and immune function (22).

Major Depressive Disorder (MDD): a mood disorder, also known as clinical depression, that lasts a period of two weeks or longer during which there is either

depressed mood or anhedonia, and at least four other symptoms that significantly impact daily functioning (24). These symptoms include hopelessness, irritability, difficulty concentrating, social withdrawal, sleep disturbances, change in appetite, musculoskeletal pain, gastrointestinal distress, and low libido (4, 6).

Monoaminergic: of, relating to, or designating monoamines that function as neurotransmitters (28).

CHAPTER 2: REVIEW OF THE LITERATURE

Introduction

Depression is a general term that encapsulates several of disorders with specific diagnostic criteria. According to the National Institute of Mental Health, major depressive disorder (MDD) can be defined as "a period of two weeks or longer during which there is either depressed mood or loss of interest or pleasure (anhedonia), and at least four other symptoms that reflect a change in functioning" (24). When symptoms of depression last for at least two years, regardless of the severity of the symptoms, the disorder can be defined as persistent depressive disorder (PDD), otherwise known as dysthmia (4). Beyond the hallmark symptoms of depressed mood and anhedonia, signs and symptoms of depression may also include hopelessness, irritability, difficulty concentrating, social withdrawal, sleep disturbances, appetite changes, and somatic complaints such as pain, psychomotor changes, and gastrointestinal distress (4, 6).

It has been estimated that 300 million individuals are affected by depression worldwide, while close to 7% of the adult population in the United States suffer from the disorder (5, 24). The implications of depression are far-reaching. Beyond its wide prevalence, depression is among the top five causes of disability globally (3). Incidence of depression has also been shown to be associated with a variety of chronic diseases such as metabolic syndrome, cardiovascular disease, diabetes mellitus, cancer, and Parkinson's disease (4, 30). It has been proposed that the association between depression and chronic disease is bidirectional, meaning that chronic disease may be a risk factor for depression, or depression may be a risk factor for the development of chronic disease (5, 30). Due to its host of associated symptoms, depression may significantly impact an individual's quality of life and ability to adequately perform in social, academic, and

professional settings. When symptoms are severe or untreated, risk of suicide increases significantly. According to the World Health Organization, approximately 800, 000 people die as a result of suicide every year (5).

Currently, a number of treatment modalities for depression exist today. Most commonly, treatment plans combine medication and psychotherapy (4). In general, antidepressant medications fall under two main categories: selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) (5). Evidence-based forms of psychotherapy for depression include cognitive-behavioral therapy (CBT), interpersonal therapy (IPT), and problem-solving therapy (4, 5). Group-based, or psychosocial therapy, has also been found to be an effective treatment modality for depression (5). In some circumstances, very severe or intractable depressive symptoms may be treated with electroconvulsive therapy (ECT), which target the vagus nerve of the neck (6, 37).

While current treatment options for depression are varied, many are associated with a number of side effects that may negatively impact quality of life and treatment compliance. For instance, side effects of SSRIs include weight gain, sleep disturbances (such as delayed onset of REM sleep, increased awakenings, and reduced total sleep), sexual dysfunction (such as decreased libido, erectile dysfunction, and anorgasmia), nausea, headaches, anxiety, and agitation (7). Further, the efficacy of both individual and combined treatment modalities for depression have been called into question. For instance, a 2012 study found that combined treatment consisting of antidepressants and psychotherapy was only slightly more beneficial than treatment with either antidepressants or psychotherapy alone. This study also found that treatment with either antidepressants or psychotherapy was not more effective at treating depression than alternative therapies such as exercise or acupuncture (17). Research has also suggested

that combined treatment modalities fail to achieve remission in greater than 30% of depressed individuals (25). Findings such as this, coupled with the risk of significant medication side effects, may explain the recent interest in alternative interventions to address depression. Interestingly, data from the 2012 National Health Interview Survey suggests that 38% of adults in the United States have used some form of a "complementary health approach" (20). Other research has indicated that use of complementary and alternative medicine (CAM) may be even more prevalent in individuals with depression and anxiety. A survey conducted two decades ago found that approximately 67% of respondents with severe depression pursued "complementary and alternative therapies" (13).

Background

Risk Factors

A relatively complex risk factor set has been identified for depression. Genetic predisposition, altered neurological function, environmental triggers, and traumatic or stressful live events are some of the most common risk factors for the disorder (5, 15). Current treatment options for depression are typically aimed at the pathophysiology associated with these factors. However, an emerging body of research has implicated additional pathways for the development of depression. In particular, recent research has focused on the associations between depression and systemic inflammation, gut-brain interactions, micronutrient deficiencies, and immune function. As the mechanisms of these associations are uncovered, a number of targeted treatment options have been proposed, many of which are nutrition-based. The purpose of this literature review is to critically investigate current research related to nutritionally relevant depression interventions.

Dietary Interventions Targeting Systemic Inflammation for Treatment of MDD

Within the last ten years, there has been a significant amount of research dedicated to investigating the relationship between biomarkers of inflammation and depression. These biomarkers include cytokines, chemokines, and acute-phase proteins such as C-reactive protein (CRP). A 2012 review published in the journal *Neuropsychopharmacology* espoused that "psychological stress, diet, obesity, leaky gut, and an imbalance between regulatory and pro-inflammatory T cells…contribute to inflammation and may serve as a focus for preventative strategies relevant to both the development of depression and its recurrence" (12).

Not surprisingly, the established anti-inflammatory properties of omega-3 fatty acids have been of interest in relation to depression. In a study published in *Brain, Behavior, and Immunity* in 2012, researchers hypothesized that omega-3 supplementation would minimize increases in proinflammatory cytokines induced by a stressful life event, and subsequently minimize feelings of anxiety and depression (18). In this double-blind randomized controlled trial, researchers divided a total of 68 medical students into 5 cohorts consisting of 9-17 students each. Exclusion criteria included, but was not limited to, high fish intake, fish and flaxseed supplementation, smoking, chronic illnesses of an inflammatory nature, lipid-altering medications, and psychoactive medications. The intervention groups received a supplement containing 2085 mg of eicosapentaenoic acid (EPA) and 348 mg of docosahexaenoic acid (DHA) for a total of 12 weeks, while the control groups received a comparable-looking capsule containing a mixture of oils in a ratio of saturated:monounsaturated:polyunsaturated fatty acids typically consumed by adults in the United States.

Data collection for all groups occurred over a roughly 28-month period during which exam periods were used as a stressful life event. There was no significant difference in age, weight, BMI, or food frequency questionnaire results between the intervention and control groups. Prior to initiation of the supplement, baseline blood samples were obtained during a low-stress period of time as well as the day preceding a major exam. Four more blood samples were taken over the course of supplementation: twice between exams and twice on the day before a major exam. The Center for Epidemiological Studies Depression Scale (CES-D) and the Beck Anxiety Inventory (BAI) were used to assess participants' depression and anxiety symptoms during the intervention. The primary outcome of the study were the cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). At the end of the study, researchers found no significant difference between serum levels of IL-6 and TNF-α. Given this, stimulated cytokine values were created by controlling for certain baseline variables.

Researchers found that omega-3 fatty acid supplementation significantly reduced stimulated IL-6 production by 14% compared to the control group (P=0.04). A 14% reduction in stimulated TNF-α was also observed although statistical significance was borderline (P=0.06). As for their secondary outcomes, researchers found that anxiety symptoms were significantly lower in the intervention group (P=0.04) while no significant difference in depressive symptoms were observed between the two groups (P=0.93) (18).

Strengths of this study include that this was randomized controlled trial with a statistical power of 90%. The authors also reported minimal attrition with 99% of the subjects completing the trial. A significant limitation of the study includes the fact that the participants did not carry formal diagnoses of MDD. Therefore, only inferences

towards the utilization of omega-3 fatty acid supplementation for the treatment of MDD can be made. Another limitation of note was that even though the researchers assessed other health-related behaviors known to affect inflammation, such as sleep hygiene, physical activity habits, and alcohol, caffeine, and tobacco use, they did not disclose whether or not these variables were controlled for during statistical analysis.

While omega-3 fatty acid supplementation did not have a beneficial impact on depressive symptoms in the previously described study, it is important to reiterate that this study evaluated symptoms of depression in otherwise healthy young adults. A study conducted by researchers at University of Navarra-Spain evaluated the effect of an anti-inflammatory diet on depression symptoms in individuals with metabolic syndrome, as both depression and metabolic syndrome are associated with elevated levels of the inflammatory biomarker CRP (30). More specifically, the researchers hypothesized that a 6-month, hypocaloric dietary pattern designed to reduce the signs and symptoms of metabolic syndrome would subsequently reduce symptoms of depression. A subsample of 60 subjects from the RESMENA-S study were used in this longitudinal study. Exclusion criteria included, but was not limited to, current antidepressant treatment and vitamin and mineral supplementation. Both the control and intervention groups followed a comparably energy-restricted diet of approximately 70% of participants' estimated energy requirements. However, the unique components of the intervention diet were patterned after the Mediterranean diet and included higher protein intake (30% of total calories), higher meal frequency, higher antioxidant capacity, and lower glycemic index foods compared to the control group diet. The primary biochemical outcome was the inflammatory marker plasma CRP. The Beck Depression Inventory (BDI), a 21-question self-report inventory, was used to assess depression symptoms at baseline, two months

into the study, and at post-intervention. The method used for assessing diet compliance was not detailed in this article although the authors referenced prior literature on the RESMENA study for further details. A brief review of this literature suggests that analysis of a 48-hour weighed food record at the end of the study was used to confirm that energy intake was consistent with the intervention dietary pattern (20).

After 6 months of treatment, researchers found no significant differences in any of the variables analyzed between the two groups, which led researchers to combine the control and intervention groups so that they could be "analyzed together as a unique experimental group" (30). Researchers also found that 6.6% of participants had a BDI score of ≥10 (indicating moderate depression) upon completion of the energy-restricted diet, compared to 25% at baseline. It was also discovered that a greater reduction in weight led to a greater reduction in depressive symptoms ($P<0.013$). A positive association between total changes in body fat and changes in depression was also observed, in which a greater loss of body fat was associated with a greater decrease in BDI score ($R^2=0.23$, $P=0.004$). Finally, even after adjusting for changes in body fat, a statistically significant positive association between BDI scores and CRP levels persisted ($P=0.015$). Of note, the mean baseline level of CRP for the combined group was 4.3 mg/L (±0.7) indicating the presence of moderate to severe systemic inflammation. At six months, the mean CRP was 2.5 mg/dL (±0.4), indicating reduced but ongoing systemic inflammation. Nonetheless, the researchers concluded that both weight loss and a reduction in CRP result in a reduction in depressive symptoms in individuals with metabolic syndrome.

Strengths of this study include the fact that it was designed to have a statistical power of 90% and that the researchers thoroughly assessed a wide range of relevant variables at multiple time-points (baseline, 2 months, and 6 months). However, a fairly significant number of study limitations were evident. The researchers provided limited discussion related to dietary compliance, revealing only that the energy intake of the combined treatment group was consistent with their prescribed dietary pattern. Similarly, description of overall study design was minimal, in which readers were referred to pre-existing literature on the larger RESMENA study. It is also important to note that while BDI scores of participants were elevated at baseline, the researchers did not disclose the number of participants that carried formal MDD diagnoses at baseline.

Similar to the RESMENA study, the large, multicenter, randomized controlled trial known as the PREDIMED study used a sub-sample population to investigate potential associations between the Mediterranean diet and depression (35). More specifically, rather than assess the impact that this dietary pattern had on depressive symptoms, as was the purpose of the RESMENA study, this trial sought to examine the effects of the Mediterranean diet on the risk of developing depression. A total of 7,447 subjects were originally recruited from 2003 to 2009 from a total of 11 hospitals throughout Spain to participate in the primary study designed to investigate the role of the Mediterranean diet in the primary prevention of CVD. Inclusion criteria for the primary study included men aged 55-80, women aged 60-80, diagnosis of DM and/or the presence of at least three CVD risk factors such as current smoking habit, HTN, elevated LDL, and low HDL. Exclusion criteria for the sub-sample included a history of depression, history of antidepressant medication use, and participants with <3 years of follow-up data from the primary study. Ultimately, a sub-sample of 3,923 subjects were randomly assigned to

one of the three intervention groups from the primary study: Mediterranean diet plus extra-virgin olive oil (MD+EVOO), Mediterranean diet plus nuts (MD+nuts), and low fat diet (LF). The LF diet functioned as the control group in this secondary study.

Participants in the MD+EVOO group were provided with one liter of EVOO per week and the participants in the MD+nuts group were provided with 30 grams per day of mixed nuts (15 g walnuts, 7.5 g hazelnuts, 7.5 g almonds). Both intervention groups received comprehensive, RD-led diet education related to the MD, which was provided as individual and groups sessions at the baseline visit and every 3 months throughout the study. A validated questionnaire was completed at each session to assess diet adherence and to guide individual dietary advice. The main dietary recommendations for the MD included, but were not limited to: 1) liberal use of olive oil, 2) increased intake of fruits, vegetables, legumes and fish, 3) decreased total meat consumption with an emphasis on reducing red and processed meats, 4) use of a homemade tomato sauce, 5) avoidance of butter, cream, fast food, concentrated sweets and sugar-sweetened beverages, and 6) moderate consumption of red wine (for current alcohol drinkers). The LF diet group was provided with education in a comparable format, with content based on low fat diet recommendations outlined by the American Heart Association. All three groups were advised against restricting energy intake and were not specifically counseled on physical activity. After 3 years of follow-up, the MD adherence questionnaire was used to categorize participants' adherence as high, medium, or low. Subjects were followed for a total of 4 years or until the occurrence of a depression diagnosis or death. In this study, incidence of depression was defined as a diagnosis made by a physician or a report of habitual use of antidepressant medication.

At baseline, differences between the control and interventions group were observed. The control group was found to have lower physical activity, lower alcohol consumption, lower rates of marriage, and lower education levels. However, the authors did not disclose if these differences were statistically significant. By completion of the study in 2010, a total of 224 new cases of depression had been identified. Covariates included in regression analysis were: age, sex, BMI, recruiting center, smoking habits, physical activity, educational level, marital status, alcohol intake, energy intake, and prevalence of chronic disease at baseline. An inverse, albeit statistically insignificant, relationship was observed between depression and the MD+nuts group (multivariate HR=0.78; 95% CI 0.5 to 1.10). Similarly, sub-analyses revealed a 20-30% reduced risk for depression in the MD+nuts, which was statistically insignificant. No association was found between depression and combined intervention groups. Per protocol analysis revealed no significant difference in risk reduction between those participants with high MD adherence compared to those participants with low MD adherence. Perhaps most notably, when sub-analysis was restricted to participants with type 2 diabetes (DM2), a statistically significant reduction in risk for depression was observed in the MD+nuts groups compared to the control (41% relative risk reduction; 95% CI 0.36 to 0.98; P=0.04). When a similar sub-analysis was conducted on participants with DM2 in the MD+EVOO group, a stronger, but still statistically insignificant, relationship between the diet and depression risk was observed.

While the RESMENA and PREDIMED studies differ in that their interventions are targeted at primary and secondary prevention of depression, respectively, the results of both studies once again highlight the strong association between DM2, metabolic syndrome (MetS), and depression. It is important to note that the RESMENA study

specifically sought to modify inflammation in the setting of MetS to reduce depressive symptoms. Even though the PREDIMED diet utilized the same dietary pattern known for its anti-inflammatory properties, biomarkers for inflammation were not assessed. Therefore, while assumptions can be made regarding the mechanisms of action of the MD+nuts diet in reducing occurrence of depression in individuals with DM2, further comparable research that assesses biomarkers of inflammation and oxidative stress is required.

Interestingly, the authors of PREDIMED do not touch upon the anti-inflammatory properties of the MD when offering up potential explanations for the reduced risk for depression conferred upon individuals in the MD+nuts group. Instead, they highlight the results of one sub-analysis of an individual study center (Navarra) that identified significantly higher levels of BDNF, a neuroprogressive protein inversely associated with depression, in the MD+nuts group compared to the control group. Another PREDIMED sub-analysis revealed that participants in the MD+nuts demonstrated significantly lower levels of fasting glucose and insulin, as well as improved HOMA score, which may suggest that reduction in the risk for depression is due to improved metabolic function. The researchers also highlight that walnuts are a significant dietary source of serotonin. However, since serotonin levels were not assessed in this study, it cannot be determined if 15 grams of walnuts per day was able to impact participants' serotonin levels in a meaningful way.

Strengths of the PREDIMED study include the large study population, the extended intervention period, and the randomized, controlled design. The researchers' decision to incorporate two, parallel intervention groups likely allowed for more accurate assessment and critical consideration of the specific components of the MD that may be

exerting beneficial effects on the study's outcome measures. However, the results of this study are undoubtedly limited by the fact that PREDIMED was designed first and foremost to assess the role of the MD in the primary prevention of CVD. This is quite apparent in that the control group was instructed to follow a low fat diet based on guidelines defined by the American Heart Association, which may have possibly conferred a unique set of benefits on participants in the control group. In fact, researchers determined that after 6 years of follow-up, the scaled mean adherence to the MD in the control group was 9 while mean adherence in the MD groups was 10.5 (no p-value provided), which suggests that adherence to a traditionally defined low-fat diet in the control group was likely quite low. Given this, future research that compares a standard American diet (SAD) to the MD diet is undoubtedly warranted.

While the three prior studies focused largely on anti-inflammatory interventions to impact depressive symptoms in individuals without a formal diagnosis of MDD, a randomized controlled trial in Iran in 2014 investigated the impact of vitamin D supplementation on not only inflammatory markers but also markers of oxidative stress in patients with MDD (36). In the corresponding article published in *The Journal of Nutrition and Disease*, the authors highlight prior research that has proposed vitamin D may be beneficial for reducing symptoms of depression by positively impacting neurotransmitters, reducing systemic inflammation, upregulating synthesis of glutathione, and promoting calcium homeostasis in the brain. To gain further insight into these possible mechanisms, the researchers randomly divided a total of 36 participants into an intervention group that received 50,000 IU of vitamin D weekly for 8 weeks and a control group that received a placebo of comparable appearance. Inclusion criteria included an age of 18-65, a diagnosis of MDD in accordance with the Diagnostic and Statistical

Manual of Mental Disorders 4th edition (DSM-IV), and a score of at least 15 on the Hamilton Depression Rating Scale (HDRS). Exclusion criteria included, but was not limited to, a history of heart attack, chest pain, kidney stones, substance abuse, and use of dietary supplements within the last 2 months. It is important to note that all participants had serum vitamin D levels that indicated deficiency. The primary outcome of this study was once again depression symptoms assessed using BDI. Secondary outcomes were biomarkers for glycemic control (such as fasting plasma glucose, serum insulin, and HOMA-B), CRP (to assess systemic inflammation), and various markers for oxidative stress (most notably total antioxidant capacity and total glutathione).

Initially, while there was a greater increase in serum vitamin D levels in the intervention group compared to the control group upon completion of the study, there was only a trend toward a greater decrease in BDI score in the intervention group compared to the control group (P=0.06). However, upon adjusting for age and BMI, a significant difference in BDI score was observed in the intervention group compared to the control group (P=0.04). Significant positive changes in serum insulin, HOMA-B, total antioxidant capacity (TAC), and total glutathione were observed in the intervention group compared to the control group, while no significant differences were observed for serum calcium, fasting plasma glucose, and CRP. Given the results of this study, supplementation of vitamin D appears to have antidepressant properties via mechanisms that target hyperinsulinemia and oxidative stress, rather than reducing systemic inflammation.

Strengths of this study include that it was a double-blind, randomized controlled trial, that the participants' metabolic status and dietary intake was thoroughly assessed at baseline and upon completion of the trial, and that compliance was >90%. However,

several significant limitations of the study were also noted. The sample size was small at 40 participants and consisted largely of females (34 females vs. 6 males). The predominantly female population, coupled with the unique cultural attributes of the Iranian participants, prevent the results of the study from being applied to the general population. Finally, the researchers did not disclose the statistical power of the study, which ultimately calls into question the reliability of the results reported.

A multitude of studies have suggested that phytochemicals, a variety of active compounds in plant foods such as fruits, vegetables and spices, have the ability to mitigate inflammation and oxidative stress (13). While the large majority of this research is related to the inflammatory and oxidative pathways associated with chronic diseases such as cardiovascular disease and diabetes mellitus, some research has focused on the potential effects that specific phytochemicals and plant foods may have on depression. Researchers out of Perth, Australia conducted a randomized, double-blind, placebo-controlled clinical trial to investigate the efficacy of curcumin extract in reducing depressive symptoms as well as to assess the ability of various peripheral biomarkers to predict treatment response (21).

A total of 50 participants were recruited to participate in this 8-week study. Inclusion criteria included but was not limited to adults aged 18-65 years, a formal diagnosis of MDD as defined by the DSM-IV, and a score of ≥14 on the Inventory of Depressive Symptomatology ($IDS\text{-}SR_{30}$). Those candidates with other major psychiatric disorders, substance abuse issues, autoimmune disease, DM, CVD, hypertension, and asthma were excluded from the study. Participants in the intervention group were instructed to take one capsule containing 500 mcg of curcumin extract twice daily. Depressive symptom severity, the primary outcome of this study, was assessed at

baseline and 8 weeks using several self-rated questionnaires with the IDS-SR_{30} serving as the principal assessment tool. A total of 13 plasma, urine, and salivary biomarkers were selected based on their known involvement in pathways related to inflammation, oxidative stress, monoaminergic activity, and hypothalamus-pituitary-adrenal (HPA) activity. Biomarker collection was completed at baseline and 8 weeks.

At baseline, the intervention and control groups were similar in all demographic variables, with the exception of a significantly higher prevalence of medical illnesses (not otherwise specified) in the control group (P=0.02). Similarly, only one significant difference was observed in the baseline levels of plasma, urine, and salivary biomarkers between groups, in which plasma epidermal growth factor (EGF) was higher in the intervention group.

At 8 weeks, 47 of the 50 enrolled participants had completed the study. In the intervention group, treatment was associated with a significant increase in urinary substance P, or SUB-P (P=0.002), a neuropeptide associated with functions such as cardiovascular regulation, gut motility, and stress-related behavior, and urinary thromboxane B2, or Tbx-B2 (P=0.003), an arachidonic acid metabolite associated with functions such as platelet aggregation, smooth muscle contraction, and peripheral adrenal catecholamine secretion. It is important to note that these changes in biomarkers were found to be unrelated to treatment outcome. Notably, the only biomarker that was found to have a significant impact on IDS-SR_{30} score following curcumin treatment was the protein known as endothelin-1 (ET-1). More specifically, participants with high baseline ET-1 experienced a significant reduction in depressive symptom severity compared to the control ($F_{2,30}$=3.82, P=0.033; Cohen's d=1.26). The researchers propose a number of potential mechanisms to explain this interaction between ET-1 status at baseline and

treatment response. ET-1 is a protein with powerful vasoactive effects and is associated with dopamine release, blood-brain barrier permeability, free radical production, and cytokine production. Prior research has linked altered ET-1 expression with Alzheimer's disease and several inflammatory brain disorders while other studies have found that depression severity effectively predicts ET-1 elevation in individuals with CVD. Animal models have implicated ET-1 in bacterial translocation in the gastrointestinal tract. Given that a significant change in ET-1 level in the intervention group was not observed over time, the results of this study suggest that curcumin may bestow anti-inflammatory, antioxidative, and subsequently, antidepressant effects in the setting of endothelial dysfunction or altered intestinal permeability.

Strengths of this study include its randomized, controlled trial design and the comprehensive array of outcome measures assessed. However, the researchers acknowledge that the small sample size and extensive statistical analyses conducted ultimately limit the reliability and statistical power of the study. Other notable weaknesses of this protocol include the lack of biomarkers to assess blood levels of curcumin or its associated compounds in order to assess for treatment compliance as well as bioavailability.

Dietary Interventions Targeting Intestinal Dysfunction for Treatment of MDD

The impact of gastrointestinal permeability as well as the distribution of specific microbial species has been implicated in depression. It has been theorized that increased gastrointestinal permeability leads to translocation of bacteria, which subsequently induces inflammation, and ultimately promotes depression (23). Other research has suggested that specific bacteria may either exert a beneficial or deleterious influence on "neural pathways and central nervous system signaling pathways" (8).

In a randomized, double-blind, placebo-controlled trial, researchers at the Kashan University of Medical Sciences in Iran investigated the impact of daily supplementation of the probiotics *Lactobacillus acidophilus, Lactobacillus casei*, and *Bifidobacterium bifidum* on depressive symptoms, glucose homeostasis, lipid profile, inflammatory biomarkers, and biomarkers of oxidative stress in individuals with MDD (1). Exclusion criteria included, but was not limited to, <20 or >55 years of age, heart attack, chest pain, substance abuse, and use of dietary or probiotic supplements during the previous 2 months. A total of 40 participants, aged 20-55 years old, completed the 8-week intervention. The primary outcome of the study was BDI score while secondary outcomes were FPG, lipid concentrations, HOMA-IR, CRP, TAC, and glutathione (GSH).

At the end of the study, statistically significant decreases in BDI score (P=0.001), serum insulin levels (P=0.03), HOMA-IR (P=0.03), and CRP levels (P=0.03) were observed in the intervention group compared to the control group. The intervention group also demonstrated significantly increased GSH levels (P=0.02). Researchers did not observe significant differences in FPG, lipid profiles, or TAC levels between the two groups. In summary, the results of this study indicate that probiotic supplementation improved depressive symptoms, reduced inflammation and oxidative stress, and improved certain aspects of glycemic control. When discussing possible mechanisms of action, the authors highlight that depression symptoms may be reduced through microbial-mediated improvements in carbohydrate absorption, increased serum tryptophan levels, and increased production of short-chain fatty acids.

Strengths of this study include that it was a double blind, randomized controlled trial with a statistical power of 80% to detect significant differences between the

intervention and control group. The high compliance rate of >90% was also a notable strength of this study. However, similar to the study conducted by Sepehrmanesh et al, the study population was Iranian and 85% female. Given this, the results of the study cannot be applied to the general population. The short duration of the trial is also a significant limitation of the study and cannot speak to the long-term efficacy of probiotic supplementation for treatment of MDD. Finally, the multispecies formulation of the probiotic supplement used in the trial ultimately prevents conclusions from being made about specific beneficial strains of bacteria.

Cognitive reactivity is the activation of dysfunctional thought patterns in response to changes in mood (38). The presence as well as the degree of cognitive reactivity is considered a risk factor for MDD. In a randomized, triple-blinded, placebo-controlled trial, researchers at Leiden University sought to determine the impact of 4-week supplementation with a multispecies probiotic on cognitive reactivity to sad mood in healthy participants without mood disorders (38). A total of 40 participants were equally and randomly assigned to intervention and control groups. Exclusion criteria included any psychiatric or neurological disorders and family history of depression and migraine. Those participants in the intervention group received daily supplementation in the form of a freeze-dried powder of various strains of Bifidobacterium and Lactobacillus, and Lactococcus lactis. Symptoms of cognitive reactivity to sad mood were assessed using the Leiden Index of Depression Sensitivity questionnaire. Depression was assessed using the Beck Depression Inventory II (BDI-II) while anxiety was assessed using the BAI.

Researchers determined that participants in the treatment group had significantly reduced self-reported cognitive reactivity to sad mood (P=0.019). No significant differences were observed in BDI-II and BAI scores between the two groups. The

researchers suggested that while probiotic supplementation didn't directly reduce symptoms of depression and anxiety, "vulnerability to mood disorders" was reduced. In other words, participants' risk for depression and anxiety was reduced by way of decreasing cognitive reactivity to sad mood. Similar to the previously discussed study, the researchers proposed biological mechanisms for action such as increased microbiota synthesis of tryptophan and improved epithelial barrier function resulting in reduced inflammation (38).

While the most notable strength of this study was that it was a randomized, controlled trial, an array of study limitations call the quality of the study into question. Most notably, this study was only a month in duration and quite small in size (n=40) with no disclosure regarding statistical power. Further, the mean age for both the intervention and controls groups was ≤20 years while the mean BMI for both groups was ≤25 kg/m^2. Since this population was relatively young and healthy, the results of this study cannot be applied to the general MDD population.

Researchers at the University of Canterbury, New Zealand opted to narrow their focus onto two specific probiotic strains, *Lactobacillus helveticus* and *Bifidobacterium longum*, to evaluate their effectiveness as the primary treatment for low mood (33). In this double-blind, randomized, placebo-controlled trial published in the *Australian & New Zealand Journal of Psychiatry* in 2017, a total of 40 participants aged 16 or older were recruited for the study after meeting the criteria for moderate low mood based on either the Quick Inventory of Depressive Symptomatology or the Depression, Anxiety, and Stress Scale. Participants were required to be free from any psychiatric medications for at least 4 weeks. They were permitted to continue psychotherapy during the trial given that they had been actively involved in treatment for at least 6 months. Exclusion

criteria included high risk for suicide or violence, neurological disorders, renal, hepatic and cardiovascular disease, pregnancy or lactation, use of supplements with potential antidepressant effects such as St. John's Wort, 5-HTP and SAMe , and recent probiotic or antibiotic use.

During the 8-week intervention period, participants in the intervention group were instructed to take one sachet of freeze-dried, orally dispersible probiotic powder containing a total of 3 billion CFU. Participants were encouraged to take the supplement at the same time every day prior to consuming a meal. Participants in the control group were similarly instructed to take a sensorially identical, orally dispersible powder.

The primary outcome of the study, severity of low mood, was assessed using the clinician-rated Montgomery-Åsberg Depression Rating Scale (MADRS) and the Improved Clinical Global Impressions (iCGI) scale, as well as the self-rated QIDS-SR16. Additional clinician- and self-rated tools were used to further assess psychological functioning. Due to the known association between irritable bowel syndrome (IBS) symptom severity and depressive symptom severity, the researchers opted to utilize the Irritable Bowel Syndrome Symptom Severity Scale (IBS-SSS) to follow potential changes in gastrointestinal changes over the course of the intervention. All assessments were completed at baseline and at the end of the trial.

In addition to investigating the efficacy of probiotic supplementation as the primary treatment for low mood, the researchers also sought to determine if various blood biomarkers were capable of either predicting or impacting treatment response. Citing the well-established link between depression and systemic inflammation, the researchers elected to assess for changes in the inflammatory markers hs-CRP, IL-6, IL-1β, and TNF-α. Blood levels of vitamin D and brain-derived neurotrophic factor (BDNF) were also

assessed as both biomarkers have been implicated in biological pathways related to depression. All biomarkers were collected at baseline and at 8 weeks.

At baseline, 75% of study participants were found to have at least one elevated inflammation marker with no significant differences between groups. The probiotic group was found to have significantly higher history of antidepressant use ($P<0.05$) and severity of IBS symptoms at baseline ($P<0.05$). The authors reported a 97% overall adherence rate, as determined by post-trial sachet counts, with no significant difference in compliance rates between groups. Upon completion of the study, no statistically significant differences were observed for any of the psychological outcomes measured in either the initial intent-to-treat analysis or the secondary analyses, including those analyses that controlled for history of antidepressant use, season at recruitment, and severity of IBS symptoms at baseline. Similarly, no statistically significant differences were observed in the change in the levels of biomarkers between groups. Despite the lack of significant group differences for all psychological and biomarker outcomes, it was observed that one biomarker, vitamin D, acted as a moderator of treatment effect on iCGI ($P<0.05$), QIDS-SR16 ($P<0.05$), GAF ($P<0.05$) in the probiotic group. Specifically, those participants with high baseline vitamin D levels experienced greater improvements in low mood and psychological functioning compared to those participants with low baseline vitamin D levels. Although the exact mechanisms of this moderating effect are unknown, the authors point to the known immune modulating effects of vitamin D and provide the supposition that low status of this nutrient would impede or neutralize any potential immune modulating effects from probiotic supplementation.

An inherent strength of this study was its randomized, controlled trial design. The population size, while undoubtedly small, conferred a power of 80% on the study. Other

strengths of this study lie in the careful consideration of the participants' history of antidepressant use and psychotherapy, and the presence of the co-morbidity of IBS. The biomarkers that were assessed were either validated biomarkers of inflammation or had strong, established associations with depression.

Conversely, a number of study weaknesses and potential limitations were observed. The authors point out that the two particular probiotic strains utilized in this study had previously demonstrated beneficial effects on psychological functioning in animals and in healthy human subjects, but had not been previously tested for effects on symptom severity in depression or systemic inflammation. It is also important to note that variables with known associations with depression, such as BMI, body fat percentage, dietary intake, and physical activity were not measured, and subsequently not controlled for, in this study. Finally, intestinal microbiome analysis was not completed post-intervention to determine if the supplemented probiotic strains were effectively colonized in the participants.

Conclusion

When regarded as a whole, certain trends appear although resounding conclusions cannot be made. Overall, dietary and supplementation interventions designed to impact inflammation, oxidative stress, and depressive symptoms appear more effective in those populations with MDD and/or chronic disease versus healthy populations. For instance, omega-3 supplementation provided to healthy individuals resulted in decreased inflammation but did not impact symptoms of depression. On the other hand, dietary interventions designed to reduce manifestations of metabolic syndrome such as glucose intolerance, hypertension, and dyslipidemia subsequently decreased depression symptoms as well as decreased markers of inflammation. Supplementation of vitamin D

also reduced symptoms of depression in individuals with MDD. A reduction in oxidative stress, but not inflammation, was also observed. However, all participants in this study were deficient in vitamin D at baseline. A similar trend in improved outcomes in individuals with MDD versus healthy individuals was seen when supplementing with probiotics. Specifically, individuals with MDD experienced decreased depressive symptoms, decreased CRP levels, and decreased oxidative stress after probiotic supplementation. On the other hand, individuals without mood disorders did not experience improvement in depression or anxiety symptoms with probiotic supplementation.

Given this, further research is needed to determine whether interventions such as vitamin D, probiotic, and fish oil supplementation should be reserved for the treatment of formal diagnoses of depression rather than for the prevention of depression and/or the treatment of transient depressed mood, as these interventions may ultimately be superfluous or deleterious in individuals who may be at risk for MDD but are otherwise healthy. Likewise, interventions targeted to reduce inflammation, oxidative stress, and gastrointestinal dysfunction and dysbiosis need to be investigated specifically in populations with MDD for longer durations since most of the study periods did not exceed 2 months in length.

Ultimately, the results of the specific research described in this literature review do not strongly support the proposal of a specific dietary prescription for the treatment of depression. Regardless, the results do uphold the current dietary recommendations for depression such as maintaining or achieving a healthy body weight, maintaining optimal vitamin D status, and consuming a diet rich in a variety of fish, fruits, vegetables, nuts, legumes, and whole grains in order to support adequate intake of micronutrients,

antioxidants, prebiotic fiber, and omega-3 fatty acids. However, it would not be warranted to universally prescribe supplementation with omega-3 fatty acids or probiotic supplements as treatment for MDD unless further research demonstrates they are an effective intervention.

CHAPTER 3: METHODS

An evidence-analysis was conducted using the model designed by the Academy of Nutrition and Dietetics, in which a 5-step process was completed to systematically select and analyze relevant research. These five steps are: 1) Formulate the Evidence Analysis Question, 2) Gather and Classify the Evidence, 3) Critically Appraise Each Article, 4) Summarize the Evidence, and 5) Write and Grade the Conclusion Statement. The Academy of Nutrition and Dietetics' Evidence Analysis Manual served as the primary tool to guide this investigator through the evidence-analysis process.

Step 1: Formulate the Evidence Analysis Question

During the initial step of the evidence analysis process, the investigator is required to develop a focused question that is relevant to a specific area of practice, is answerable is the sense that it is able identify associations between nutritional factors and health outcomes, and is posed in the PICO format. Initially, the primary research question was such: In adults with Major Depressive Disorder (MDD), which dietary interventions are effective at reducing symptom severity by way of reducing systemic inflammation and oxidative stress? However, in order to satisfy the recommendation for questions posed in the PICO format, this primary research question was subsequently broken into two sub-problems to ensure that the evidence-analysis process remained concise and could effectively identify potential associations between specific factors and health outcomes. The final two sub-problems are as follows:

1) In adults with MDD, how effective is polyunsaturated omega-3 fatty acid supplementation at reducing depressive symptom severity compared to conventional treatment or placebo?

2) In adults with MDD, how effective is probiotic supplementation at reducing depressive symptom severity compared to conventional treatment or placebo?

Step 2: Gather and Classify the Evidence

During this step of the evidence analysis process, the investigator must develop a plan to undertake a thorough literature search. In order to effectively do this, a concise set of search terms and inclusion and exclusion criteria must be defined (Table 1). A literature search was completed using Aurora Health Care online library and PubMed. The following search terms were employed: depression and nutrition; depression and inflammation; depression and oxidative stress; depression and probiotics; depression and yogurt; depression and lactobacillus; depression and bifidobacterium; depression and fish oil; depression and polyunsaturated fatty acids; depression and fish; and depression and omega-3.

The inclusion criteria for this literature search were primary research involving humans, adult participants aged 18-75 years of age, participants that met the criteria for depression determined using a validated diagnostic tool or a clinician-administered diagnosis, interventions involving the administration of supplements or dietary sources of either omega-3 fatty acids or probiotic bacteria, and study outcomes that measured depression severity using a validated assessment tool. The exclusion criteria for the literature search were studies involving only animal subjects and those not available in English.

Table 1: Search Plan and Results

Question	In adults with MDD, how effective is polyunsaturated omega-3 fatty acid supplementation at reducing depressive symptom severity compared to conventional treatment or placebo? In adults with MDD, how effective is probiotic supplementation at reducing depressive symptom severity compared to conventional treatment or placebo?
Date of Literature Review	• September – October, 2017 • September – November, 2018
Inclusion Criteria	• Adults 18-75 • Participants that meet the criteria for depression determined using a validated diagnostic tool or a clinician-administered diagnosis • Interventions involving the administration of supplements or dietary sources of either omega-3 fatty acids or probiotic bacteria • Study outcomes that measure depression severity using a validated assessment tool
Exclusion Criteria	• Studies involving only animals • Studies not available in English
Search Databases and Terms	• Search Databases: o Aurora Health Care Online Library o PubMed • Search Terms: o depression and nutrition o depression and inflammation o depression and oxidative stress o depression and probiotics o depression and yogurt o depression and lactobacillus o depression and bifidobacterium o depression and fish oil o depression and polyunsaturated fatty acids o depression and fish o depression and omega-3
List of Articles Included from Database	• Akkasheh, G, Kashani-Poor, Z, Tajabadi-Ebrahimi, M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. Nutrition. 2016;32: 315-320. • Gertsik L, Poland RE, Bresee C, Hyman Rapaport M.

	Omega-3 fatty acid augmentation of Citalopram treatment for patients with major depressive disorder. J Clin Psychopharmacol. 2012;32(1):61-64. doi:10.1097/JCP.0b013e31823f3b5fd • Jazayeri S, Tehrani-Doost M, Keshavarz SA, et al. Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine, separately and in combination, in major depressive disorder. Aust N Z J Psychiatry.2008;42:192-198. • Kazemi A, Noorbala AA, Azam K, Eskandari MH, Djafarian K. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clincial trial. Clin Nutr. 2018;1-7. doi:10.1016/j.clnu.2010.04.010. • Lesperance F, Frasure-Smith N, St-Andre E, Turecki G, Lesperance P, Wisniewski SR. The efficacy of omega-3 supplementation for major depression: A randomized controlled trial. J Clin Psychiatry. 2011;72(8:1054-1062. • Mischoulon D, Papakostas GI, Dording CM, et al. A double-blind, randomized controlled trial of ethyl-eicosapentaenoate for major depressive disorder. J Clin Psychiatry. 2009;70(12):1636-1644.doi:10.4088/JCP.08m04603. • Mischoulon D, Nierenberg AA, Schettler PJ, et al. A double-blind, randomized controlled clinical trial comparing eicosapentaenoic acid versus docosahexaenoic acid for depression. J Clin Psychiatry. 2015;76(1)54-61. • Pinto-Sanchez MI, Hall GB, Ghajar K, et al. Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: A pilot study in patients with Irritable Bowel Syndrome. Gastroenterology. 2017;153:448-459. doi:10.1053/j.gastro.2017.05.003. • Romijn AR, Rucklidge, JJ, Kuijer, RG, Frampton, C. A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. Aust N Z J Psychiatry. 2017;51(8):810-821. doi:10.1177/0004867416686694. • Rudzki L, Ostrowska L, Pawlak D, et al. Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improved cognitive function in patients with major depression: A double-blind, randomized, placebo controlled study.Psychoneuroendocrinology. 2018;100:213-222. doi:10.1016/j.psyneuen.2018.10.010.
List of Excluded Articles with Reason for Exclusion	• Kiecolt-Glaser, J. K., Belury, M.A., Andridge, R., Malarkey, W.B., & Glaser, R. (2011). Omega-3 supplementation lowers inflammation and anxiety in medical students: A randomized controlled trial. *Brain,*

	Behavior, and Immunity, 25 (8), 1725-1734. o Healthy subjects • Lopresti, A., Maes, M., Meddens, M.J.M., Maker, G.L., Arnoldussen, E., & Drummond, P.D. (2014). Curcumin and major depression: A randomized, double-blind, placebo-controlled trial investigating the potential of peripheral biomarkers to predict treatment response and antidepressant mechanisms of change. European Neuropsychopharmacology, 25, 38-50. o Intervention did not involve omega-3 fatty acids or probiotics. • Marangell, LB, Martinez, JM, Zboyan HA, Kertz B, Kim HFS, Puryear LJ. A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. Am J Psychiatry. 2003;160:996-998. o Research study published as a brief report. • Mohammadi AA, Jazayeri S, Khosravi-Darani K, et al. The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers. Nutr Neurosci. 2016;19(9): 387-395. o Healthy subjects (MDD/depressive symptoms not diagnosed/assessed at screening). • Perez-Cornago, A., de la Iglesia, R., Lopez-Legarrea, P., Abete, I., Navas-Carretero., S., Lacunza, C., … Zulet, M.A. (2014). A decline in inflammation is associated with less depressive symptoms after dietary intervention in metabolic syndrome patients: A longitudinal study. *Nutrition Journal*, 13 (36) , 1-9. o Subjects with metabolic syndrome (MDD/depressive symptoms not diagnosed/assessed at screening) • Sanchez, M, Darimont C, Panahi S, Drapeau V, et al. Effects of a diet-based weight-reducing program with probiotic supplementation on satiety efficiency, eating behavior traits, and psychosocial behaviours in obese individuals. Nutrients. 2017;284: 5-17. o Healthy, obese subjects. • Sanchez-Villegas, A., Martinez-Gonzalez, M.A., Estruch, R., Salas-Salvado, J., Corella, D., Covas, M.I., …Aros, F. (2013). Mediterranean dietary pattern and depression: the PREDIMED randomized trial. *BMC Medicine*, 11(208), 1-20. o Healthy subjects; intervention did not involve omega-3 fatty acids or probiotics. • Slykerman RF, Hood F, Wickens K, et al. Effect of Lactobacillus rhamnosus HN001 in pregnancy on postpartum symptoms of depression and anxiety: A randomized double-blind placebo-controlled trial. Ebiomedicine. 2017; 24:159-165. o Pregnant women (MDD/depressive symptoms not diagnosed/assessed at screening). • Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J., & Colzato, L.S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive

	reactivity to sad mood. *Brain, Behavior, and Immunity*, 48, 258-264. o Healthy subjects
Summary of Articles Identified to Review	• Summary of Articles Identified to Review: o Included primary research articles identified: >50 o Number of articles considered but excluded: 9 o Total number of included articles: 10

Step 3: Critically Appraise Each Article

The purpose of this step is to critically review each article and extract key information that will ultimately be used to summarize the study and assign it an overall rating of positive, neutral, or negative. After carefully reading each article, this investigator used the Academy's *Evidence Worksheet* to capture critical information regarding study design, major findings and statistical results, the researcher's published conclusion, and this investigator's comments related to study limitations and applicability. The Academy's *Quality Criteria Checklist*, which poses a total of 14 yes/no questions related to relevance and validity, was used in tandem to the *Evidence Worksheet* in order to aid this investigator in identifying key information to extract. The *Quality Criteria Checklist* was also used to assign an overall rating to each article.

Step 4: Summarize the Evidence

The purpose of this step is to organize, summarize, and synthesize the body of research that has been critically reviewed. First, this investigator employed the Academy's *Overview Table Template* to compile the key comparison factors of all the reviewed articles, such as study design, study population, interventions, outcomes, and limitations. Next, this investigator wrote a brief but concise summary of only those articles with findings relevant to the research question. As determined by the Academy,

each summary included the author(s), publication year, outcomes/measurements of interest, important sample characteristics and comparison factors, implications for practice, and limitations of findings. Finally, this investigator composed the *Evidence Summary*, in which patterns and commonalities in the research were highlighted and discussed, and an overall summary statement was provided.

Step 5: Write and Grade the Conclusion

The final step of the evidence analysis process involves the creation of a concise conclusion statement that synthesizes the information extracted from each article, emphasizing the articles of higher quality. Finally, this investigator assigned a grade to the conclusion statement in order to indicate the strength, or lack thereof, of the evidence provided in the body of research.

CHAPTER 4: RESULTS

The current research related to supplementation of probiotics and omeg-3 fatty acids for treatment of depression is highly varied in regards to population, intervention, and outcomes of interest. In particular, a large majority of relevant studies often focus on healthy subjects that do not carry formal diagnoses of MDD. Subsequently, while the endpoints of these studies may include depressive symptom severity, the results cannot be appropriately translated into evidence-based practice guidelines for the treatment of MDD. Given this, the research on probiotics and omega-3 supplementation specifically related to populations with MDD are quite limited. For this systematic review, a total of five studies were critically analyzed for each of the two research questions. All studies involved adult participants that met clinical criteria for depression at baseline and measured depressive symptoms using a validated tool as a primary study outcome. Each study was critically and systematically appraised using a data extraction worksheet and *Quality Criteria Checklist*. An individual overview table for each group of studies can be located in Appendix A.

Relevant Research Findings

Omega-3 Fatty Acid Supplementation

Gertsik et al., 2012. Rating: +

In this randomized controlled trial published in 2012, researchers sought to determine whether supplementation of omega-3 fatty acids would improve treatment efficacy and accelerate response to the antidepressant medication citalopram. A total of 42 adult participants meeting DSM-IV criteria for MDD were initiated on 20 mg/day of citalopram. Those individuals in the treatment group were also instructed to take two capsules of omega-3 fatty acids twice daily for a total 8 weeks. Daily supplementation

provided a total of 1800 mg EPA, 400 mg DHA, and 200 mg of miscellaneous omega-3 fatty acids. Citalopram dose was increased to 40 mg/day as needed at study week 4. Depressive symptoms were measured at baseline and at study weeks 2, 4, 6, and 8 utilizing the clinician-administered Hamilton Depression Rating Scale (HAM-D) as the primary study outcome. Additionally, BDI, MADRS, CGI, and PGI were measured at baseline and at study weeks 2, 4, 6, and 8, and plasma CRP and 24-hour urinary cortisol were collected at baseline and study completion. After controlling for citalopram dose during statistical analysis, a significant improvement in HAM-D score was observed at study completion in the omega-3 group compared to the placebo group (P=0.006). Additionally, significant differences in HAM-D score in the omega-3 group compared to placebo group were also observed at week 4 (P=0.018) and at week 6 (P=0.007). However, the lack of significant group differences at week 2 indicate that omega-3 supplementation did not accelerate response to citalopram. Remission status, with full remission defined as a HAM-D score of ≤7 and partial remission defined as improvement of HAM-D ≥50% (for final scores of 8-15), was also significantly improved in the omega-3 group (P=0.018). No significant changes in plasma CRP or 24-hour urinary cortisol were observed between groups at study completion, providing no meaningful insight into the mechanistic action of the beneficial effects of omega-3 supplementation that were observed. Limitations of this study include the small sample size and the minimal subject demographic data disclosed by the authors.

Jazayeri et al., 2008. Rating: +

Jazayeri and colleagues conducted a randomized controlled trial to compare the effects of monotherapy with EPA supplementation, monotherapy with fluoxentine, and combination therapy with EPA supplementation plus fluoxetine for treatment of MDD.

A total of 48 adult participants were enrolled in this 8-week, 3-arm study. All participants had been free from antidepressant medication for at least 6 weeks at study initiation. Those participants receiving monotherapy with EPA supplementation received two capsules daily for a total of 1100 mg ethyl-EPA per day. Individuals assigned to the fluoxentine group were maintained on 20 mg/day for the duration of the study. Those participants receiving combination therapy received 1100 mg of ethyl-EPA plus 20 mg/day of fluoxetine throughout the course of the study. The Hamilton Depression Rating Scale (abbreviated as HDRS by the authors) was used as the primary endpoint to assess depressive symptom severity and was measured at baseline and study weeks 2, 4, 6, and 8. Statistical analysis was conducted utilizing ANCOVA with baseline HDRS score, age of depression onset, and number of previous depressive episodes used as covariates. Combination therapy with EPA supplementation plus fluoxetine was found to be more effective at reducing HDRS score at 8 weeks compared to both monotherapy groups (P=0.005 for combination therapy compared to fluoxetine monotherapy; P=0.009 for combination therapy compared to EPA monotherapy). Response rates, defined as a total reduction in baseline HDRS score of >50%, were 50% in the fluoxetine group, 56% in the EPA group, and 81% in the combination group. There was no significant difference in HDRS score at 8 weeks between the monotherapy groups (P=0.426). While the comparable efficacy between monotherapies could speak to the potential of using EPA supplementation in lieu of antidepressant medication, the authors of the study point to the possibility that neither EPA nor fluoxetine are more effective than no treatment. The small sample is the primary limitation of this study.

Lesperance et al., 2011. Rating: +

Lesperance et al sought to determine whether an EPA-rich omega-3 supplement was more effective than placebo at reducing depressive symptoms in a depressed population with heterogeneous antidepressant use. A total of 432 adults with a diagnosis of major depressive episode based on the Mini-International Neuropsychiatric Interview (MINI) were enrolled in the study. Participants were required to have been on a maximum tolerated dosage of antidepressant medication for greater than 4 weeks, or if antidepressant therapy was not in place at screening, were required to have been intolerant to at least 2 antidepressant medications or refused medication therapy against medical advice. Those participants in the intervention arm of the study were instructed to take three capsules of EPA-rich omega 3 fatty acids (comprised of 70% EPA and 5% DHA ethyl esters) to provide the equivalent of 1050 mg EPA and 150 mg DHA daily for 8 weeks. Participants were encouraged not to modify their usual diet during the duration of the study. Depressive symptoms were measured using the Inventory of Depressive Symptomatology Self-Report (IDS-SR) as the primary endpoint and the Montgomery-Åsberg Depression Rating Scale (MADRS) as the secondary endpoint. Both IDS-SR and MADRS were measured at baseline and study weeks 1, 2, 4, and 8. Statistical analysis was performed on an intent-to-treat basis using mix-effect regression models. Despite adjusting for baseline IDS-SR and MADRS scores, adjuvant antidepressant use, and study site, only a trend towards improved depressive symptoms was observed in the omega-3 group compared to the placebo group (P=0.088 for mean IDS-SR; P=0.053 for mean MADRS score). Effect size of treatment was noted to be small (0.11 for IDS-SR and 0.10 for MADRS). Exploratory analyses revealed that EPA supplementation conferred a greater improvement in depressive symptoms when comorbid anxiety was

not present, in which the mean adjusted difference in IDS-SR score was 3.17 points (P=0.007; 0.27 effect size) and mean difference in MADRS score was 1.93 (P=0.008; 0.26 effect size). While this study does not provide evidence for the efficacy of EPA-rich supplementation in reducing depressive symptoms in the setting of heterogeneous antidepressant use, it does demonstrate efficacy in those individuals without comorbid anxiety, and supports the need for further research that compares the efficacy of omega-3 supplementation in these two distinct MDD profiles. Limitations of this study include the heterogeneous use of antidepressant medication and the lack of parameters related to remission or response rates, which ultimately prevent determinations from being made on whether or not improvements in IDS-SR or MADRS were of clinical significance.

Mischoulon et al., 2009. Rating: +

The efficacy of EPA supplementation as a stand-alone treatment for MDD was examined in a randomized controlled trial designed by Mischoulon et al. Forty-one adult participants with MDD as determined by the Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition (SCID-I/P), baseline HDRS score of ≥18, and a Clinical Global Impression-Severity Scale (CGI-S) of ≥3 were enrolled in the study. All participants were free from antidepressant medication at the time of enrollment. Participants in the EPA group were instructed to take a total of two capsules of 550 mg of ethyl-ester EPA daily for a total of 8 weeks. Participants were encouraged not to modify their usual diet during the duration of the study. Change in HDRS score was the primary outcome of the study and was measured at baseline and every 2 weeks for a total of 8 weeks. Clinical response was defined as final HDRS score of ≤7. Plasma levels of EPA, DHA, total omega-3, total omega-6, and omega-6/omega-3 ratio were secondary study endpoints and were measured at baseline and at study completion. Completer and intent-

to-treat (ITT) analyses were conducted on data for participants with at least 1 post-baseline evaluation. In the completers sample (n=24), there was a trend towards greater improvement of HDRS score in the EPA group compared to the placebo group (P=0.087) but no significant difference in remission or response rates was observed between groups. Similarly, there was no significant difference in HDRS score, remission, or response rates between groups in the ITT sample. Additional analyses revealed a significant association between baseline omega-6/omega-3 ratio and decrease in HDRS score (P=0.03) as well as treatment response (P=0.32) in the EPA group, suggesting that omega-3 supplementation may be more appropriate for those individuals with a suboptimal baseline ratio of omega-6 to omega-3 fatty acids. Ultimately, this study does not support EPA supplementation as a stand-alone treatment of MDD but points to the need for further research to clarify the relationship of omega-6/omega-3 ratio to both depression pathophysiology and treatment. Weaknesses of this study include a small sample size with limited statistical power and the lack of comparison of the participants' baseline characteristics between groups.

Mischoulon et al., 2015. Rating: +

The purpose of this randomized controlled trial was to compare the efficacy of EPA-rich and DHA-rich supplements as monotherapy for MDD. A total of 196 adults met the inclusion criteria, including a diagnosis of MDD per the SCID I/P, baseline HDRS score of ≥15, and CGI-S score of ≥3. Concurrent use of psychotropic medications was prohibited, as well as any trial of 40 mg/day citalopram (or equivalent antidepressant) lasting six weeks or more during the current depressive episode. Participants in the treatment groups either received two daily capsules of an EPA-enriched omega-3 supplement (for a total 1000 mg/day of EPA) or four daily capsules of

a DHA-enriched omega-3 supplement (for a total of 1000 mg/day of DHA) for 8 weeks. A double-dummy placebo design was used to maintain the blind. Participants were encouraged not to modify their usual diet during the duration of the study. The severity of depressive symptoms, as measured by HDRS, was the primary outcome of the study and was assessed at baseline and every 2 weeks throughout the study. Secondary endpoints included CGI-S, Clinical Global Impression Scale-Improvement (CGI-I), the 16-item Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR-16), Well-Being Scale (WBS), and Quality of Life Satisfactions Questionnaire (Q-LES-Q), and were also measured at baseline and every 2 weeks throughout the 8-week study. Statistical analysis was performed on a modified ITT basis. Each of the 3 study groups demonstrated a significant improvement in HDRS, QIDS-SR-16, CGI-S, Q-LES-Q, and WBS scores with effect sizes failing to demonstrate a superior treatment. There was no significant difference in remission rates (approximately 30% across groups) or response rates (40-50% across groups) between the groups. In summary, neither EPA- nor DHA-enriched omega-3 supplements were more effective than placebo as monotherapy for depression. A key limitation of this study was that the sample size did not meet requirements for statistical power, which precludes any conclusions.

Probiotic Supplementation

Akkasheh et al., 2016. Rating: +

Researchers at the Kashan University of Medical Sciences in Iran investigated the impact of daily supplementation of the probiotics L. acidophilus, L. casei, and B. bifidum on depressive symptoms, glucose homeostasis, lipid profile, and biomarkers of inflammation and oxidative stress in individuals with MDD. A total of 40 participants meeting the criteria for MDD as defined by the DSM-IV with an HDRS score of ≥15

completed this 8-week intervention. Daily probiotic supplementation was in the form of one capsule containing 2 billion CFU of each bacteria species. The primary outcome of the study was severity of depressive symptoms as measured by BDI score, while secondary outcomes were FPG, lipid concentrations, HOMA-IR, CRP, TAC, and GSH. At the end of the study, statistically significant decreases in BDI score (P=0.001), serum insulin levels (P=0.03), HOMA-IR (P=0.03), and CRP levels (P=0.03) were observed in the probiotic group compared to the placebo group. A significant increase in GSH level (P=0.02) was also observed in the probiotic group compared to placebo. While some of the proposed beneficial effects of probiotic supplementation on mood are the modulation of inflammatory, oxidative, and glycemic pathways, the findings of Akkasheh et al have been the only results of this particular evidence analysis to provide evidence to support these mechanisms. Limitations of this study include the lack of control for antidepressant use and the small and predominantly female sample population, which prevents the results of this study from being generalized to the larger MDD population.

Kazemi et al., 2018. Rating: +

Kazemi et al designed a randomized controlled trial to investigate the effects of both probiotic and prebiotic supplementation on depressive symptoms in a group of adults receiving antidepressant medications. Study participants met clinical criteria (not disclosed) for mild to moderate depression and were required to have received sertraline, fluoxetine, citalopram, or amitriptyline for at least 3 months prior to the beginning of the study. A total of 110 participants were stratified by age (<35 vs ≥35) and randomly assigned to either the probiotic treatment group, prebiotic treatment group, or placebo group. Over the course of 8 weeks, participants in the probiotic group took one sachet per day of an orally dispersible powder containing 10 billion CFU of freeze-dried L.

helveticus R0052 and B. longum R0175 while participants in the prebiotic took one sachet per day of an orally dispersible powder containing galactooligosaccharide. Depressive symptoms, as measured by BDI score, were measured at study completion. Serum levels of kynurenine:tryptophan ratio (KYN:TRP) and tryptophan:branch chain amino acids ratio (KYN:BCAAs), the secondary outcomes of the study, were also collected at baseline and study completion. Statistical analysis of BDI score was completed on an ITT basis while KYN:TRP and TRP:BCAAs was analyzed on a per protocol (PP) basis. Treatment with the probiotic supplement resulted in a significant improvement in mean BDI score compared to the prebiotic and placebo groups (P=0.042). After controlling for serum isoleucine, a significant decrease in KYN:TRP ratio was observed in the probiotic group compared to placebo (P=0.048). According to the study authors, this particular finding provides further evidence that probiotic species decrease the activity of enzymes involved in tryptophan degradation in the KYN pathway in the GI tract, which ultimately increases tryptophan availability for serotonin synthesis. Despite the significant improvement in depressive symptoms observed with probiotic supplementation, the clinical significance of these improvements is unclear due to the lack of parameters measuring remission or response rates.

Pinto-Sanchez et al., 2017. Rating: +

Due to the well-established association between IBS and psychiatric comorbidity, a sample of IBS patients with depression and anxiety were the focus of an intervention to examine the effect of B. longum supplementation on a wide variety of endpoints, including affective symptoms, IBS symptoms, inflammatory biomarkers, and HPA axis function. All participants enrolled in this randomized controlled trial (n=44) had baseline scores of 8-14 on the Hospital Anxiety and Depression (HAD) scale. Concurrent use of

antidepressant medication was prohibited. The intervention consisted of daily supplementation with a powder containing 10 billion CFU of spray-dried B. longum NCC3001, which was consumed in 100-200 mL of lactose-free milk, soy milk, or rice milk that had been heated to 20°C. A reduction of depression and/or anxiety score of ≥2 points on the HAD-D (depression) and HAD-A (anxiety) scales was the primary outcome of the study and was measured at baseline, 6 weeks, and at 10 weeks, and analyzed on an ITT basis. At 6 weeks, 14 participants in the probiotics group had HAD-D depression scores that had improved by ≥2 points compared to 7 participants in the placebo group (RR 1.98, CI 1.16-3.38, P=0.04). In PP analysis, 78% of participants in the probiotics group, compared to 35% of participants in the placebo group, had lower depression scores at 6 weeks (RR 2.4, CI 1.26-4.58, P=0.016). At 10 weeks follow-up, improvement in HAD-D scores was sustained in ITT analysis (R 2.05, CI 1.07-3.93, P=0.04) and PP analysis (RR 2.14, CI 1.11-4.12, P=0.04). A sensitivity analysis revealed that probiotic supplementation was more likely to confer an improvement in depression symptoms in those participants who also reported adequate relief of IBS symptoms (P=0.03 at 6 weeks; P=0.04 at 10 weeks). A description of the findings related to other endpoints such as brain activation patterns and inflammatory markers can be found in the corresponding Evidence Analysis Worksheet in Appendix B. A key limitation of this study is that the participants lacked a clinician-administered diagnosis of MDD.

Romijn et al., 2017. Rating: +

Daily supplementation with an orally dispersible powder containing at least 3 billion CFU of L. helveticus R0052 and B. longum R0175 for a total of 8 weeks was the intervention utilized in this randomized controlled trial conducted by Romijn et al to test for potential effects on depressive symptoms. Participants (n=79) were required to be

free of any psychiatric medications for at least 4 weeks and to have a baseline score of ≥11 on the QIDS-SR16 and a score of ≥14 on the DASS-42. The primary outcomes of this study were the MADRS, Improved Clinical Global Impressions Scale (iCGI), and the QIDS-SR16, which were measured at baseline and every 2 weeks throughout the study. Secondary endpoints of the study included the Global Assessment of Functioning (GAF), DASS-42, Irritable Bowel Syndrome Symptom Severity Scale (IBS-SSS), and serum IL-1β, IL-6, hsCRP, TNF-α, vitamin D, and BDNF. Initial intent-to-treat analysis as well as secondary analyses that controlled for factors such as history of antidepressant use, season of recruitment, and presence of IBS and severity of symptoms, showed no significant group differences or effect on any outcome measure. The only finding of note was observed with an exploratory analysis involving vitamin D status of participants, in which baseline vitamin D status had a significant, moderating effect on iCGI-S, QIDS-SR16, and GAF ($P<0.05$) in the probiotic group. Those individuals in the probiotic group with the highest baseline vitamin D levels demonstrated the greatest improvement in the aforementioned psychological outcomes compared to those individuals in the probiotic group with low baseline vitamin D levels. Authors of the study suggested that the immune-modulating properties of vitamin D might play a critical role in response to probiotic treatment. Of note, dietary sources of probiotic bacteria and relevant anthropometric variables such as BMI were not measured or controlled for, which is a potential limitation of this study.

Rudzki et al., 2018. Rating: +

The bacteria L. Plantarum 299v was the probiotic of interest in a randomized controlled trial involving 79 depressed patients receiving concurrent treatment with an SSRI. At baseline, all participants met the criteria for MDD as defined by DSM-IV-R

and were either receiving SSRI monotherapy or were free from antidepressants. Those participants not already receiving SSRI therapy were subsequently started on the medication upon study initiation. Participants in the probiotic group ingested two supplement capsules daily, each containing 10 billion CFU of L. Plantarum 299v, for a total of 8 weeks. The primary study endpoints were depression and anxiety and were measured at baseline and study weeks 4 and 8 using the HAM-D 170, Symptom Checklist (SCL-90), and the Perceived Stress Scale (PSS-10). A variety of biochemical biomarkers, including cortisol plasma concentrations, TNF-α, IL-6, and IL-1β, were collected at baseline and week 8. A full list of the cognitive function tests that were included as endpoints can be located in the corresponding Evidence Analysis Worksheet in Appendix B. Statistical analysis was completed on a PP basis and revealed no significant changes in depression or anxiety symptoms in either group. Post hoc analysis demonstrated significant improvements in the probiotic group compared to placebo for Work Speed in the Attention and Perceptivity Test ($P<0.001$) and the California Verbal Learning Test ($P=0.024$), indicating a degree of improved cognitive function, as well as significant improvements in KYN concentration ($P=0.017$) and 3HKYN:KYN ratio ($P=0.015$), suggesting possible amelioration of pathways implicated in the pathophysiology of depression.

Conclusion Statements

Research Question 1:

In adults with MDD, how effective is polyunsaturated omega-3 fatty acid supplementation at reducing depressive symptom severity compared to conventional treatment or placebo?

Conclusion Statement

When regarded as a whole, the research suggests that supplementation of polyunsaturated omega-3 fatty acids is not more effective at reducing depression symptoms severity than conventional treatment or placebo. Rather, the use of EPA-rich omega-3 supplementation as therapy adjuvant to treatment with antidepressant medication appears to be more effective than conventional treatment. Two studies evaluated the effectiveness of combination therapy comprised of a daily omega-3 supplement plus an SSRI. Gertsik et al. found that daily supplementation with an omega-3 supplement that provided a total of 1800 mg/day EPA and 400 mg/day of DHA, combined with 20-40 mg/day of citalopram significantly reduced depression symptoms as measured by HAM-D score (P=0.008). Similarly, Jazayeri et al. compared EPA-rich omega-3 monotherapy (1100 mg EPA daily), fluoxetine monotherapy, and EPA-rich omega-3 plus fluoxetine combination therapy, and found that combination therapy was significantly more effective at reducing depression symptoms as measured by HDRS score (P=0.005 fluoxetine compared to combination; P=0.009 EPA compared to combination). Conversely, Mischoulon et al. (2009) found that daily EPA-rich omega-3 supplementation (providing 1000 mg/day EPA) did not confer significant improvements in depressive symptoms when used as a stand-alone treatment for depression. Lesperance et al. elected to not control for antidepressant use when testing the effectiveness of daily supplementation with an EPA-rich omega-3 supplement (1050 mg/day EPA, 150 mg/day DHA) and ultimately observed only a nonsignificant trend towards improved depressive scores, as measured by IDS-SR (P=0.088) and MADRS (P-0.053), in the intervention group. Mischoulon et al. (2015) later compared the effectiveness of a daily EPA-rich omega-3 supplement (1000 mg/day) to a daily DHA-rich omega-3 supplement (1000 mg/day) and found that neither form of monotherapy was

more effective at reducing depressive symptoms than placebo. In summary, current research supports daily supplementation with at least 1100 mg EPA in combination with SSRI for the treatment of MDD.

Grade: II, Fair

Research Question 2:

In adults with MDD, how effective is probiotic supplementation at reducing depressive symptom severity compared to conventional treatment or placebo?

Conclusion Statement

The research does not currently provide adequate evidence that probiotic supplementation is more effective than conventional treatment or placebo at reducing depressive symptom severity in individuals with MDD, although dose and probiotic species likely plays a critical role in efficacy. Romijn et al. found that daily supplementation of approximately 3 billion CFU of L. helveticus plus B. longum was not effective as monotherapy for MDD, although poor vitamin D status may have diluted treatment response. Conversely, Pinto-Sanchez et al. found that daily supplementation with 10 billion CFU of B. longum was more effective at reducing HAD-D scores than placebo in a sample of depressed subjects not receiving concurrent antidepressant therapy (P=0.04). Rudzki et al. tested the effectiveness of daily supplementation with 20 billion CFU of L. plantarum on a sample of depressed adults receiving either new or ongoing treatment with an SSRI and observed no significant improvement in depressive symptoms in the probiotic group compared to placebo. In contrast, Akkasheh et al. elected to not control for antidepressant use and found that daily supplementation with approximately 6 billion CFU of L. acidophilus, L. casei, and B. bifidum was more effective at reducing depressive symptoms than placebo (P=0.001). Kazemi et al. was the

only study in which antidepressant use was required at enrollment. The results of this study indicate that combination therapy with antidepressant medication plus daily supplementation of 10 billion CFU of L. helveticus plus B. longum was more effective at reducing depressive symptoms than conventional treatment with SSRIs or tricyclic antidepressants alone (P=0.042). In summary, although current research does not strongly suggest that probiotic supplementation is more effective as a monotherapy, the results of this evidence analysis suggest that a supplement containing either Bifidobacterium or a multispecies probiotic in a dose of at least 6 billion CFU may be more effective at reducing depressive symptoms than conventional treatment or placebo.

CHAPTER 5: EVIDENCE SUMMARY AND DISCUSSION

Omega-3 Supplementation for Treatment of MDD

Evidence Summary

All five studies included in this evidence analysis utilized adult populations with either a formal diagnosis of MDD, as defined by the DSM-IV, or the presence of depressive symptoms confirmed by a validated tool, at screening. Overall, each study imposed relatively similar inclusion and exclusion criteria that controlled for serious comorbid medical conditions, pregnancy and lactation, psychotic disorders, and suicidal/homicidal ideation. All five studies used depressive symptom severity as the primary study outcome with four of the five studies measuring these symptoms using the 17-item, clinician-administered Hamilton Depression Rating Scale (HDRS, also known as HAM-D). The fifth study, conducted by Lesperance et al., elected to assess change in depressive symptoms using both the 30-item Inventory of Depressive Symptom Self-Report (IDS-SR) and the clinician-administered Montgomery-Åsberg Depression Rating Scale (MADRS). All five studies utilized intervention periods of 8 weeks.

When regarded as a whole, the research supports use of omega-3 supplementation in combination with conventional antidepressant medication for treatment of MDD. The studies conducted by Gertsik et al. and Jazayeri et al. suggest that omega-3 supplements with an EPA content of 1100-1800 mg/day, when given in combination with an SSRI, are more effective at reducing depressive symptoms compared to conventional monotherapy with a SSRI. Conversely, the two studies conducted by Mischoulon et al. in 2009 and 2015, which investigated omega-3 supplementation as stand-alone treatment, did not demonstrate significant improvements in depressive symptoms. Interestingly, Lesperance et al. utilized a population with heterogeneous use/nonuse of antidepressants

with the intervention group demonstrating a nonsignificant trend towards greater improvement in depressive symptoms.

Form and Dosage

Although the results of these studies suggest that omega-3 supplementation may only be beneficial as combination therapy, the inconsistency in composition and dosage of omega-3 fatty acids must be considered. Gertsik et al. found omega-3 supplementation combination therapy to be effective at a dose of 1800 mg EPA plus 400 mg DHA. On the other hand, Jazayeri et al. observed favorable effects with a combination therapy incorporating 1100 mg of EPA only. Conversely, Lesperance et al. employed a dose of 1050 EPA plus 150 mg DHA without significant results, while Mischoulon utilized 1000 mg EPA in their 2009 study and compared 1000 mg EPA to 1000 mg DHA in their 2015 study without favorable results.

Study Strengths and Limitations

All five studies were randomized controlled trials and were generally well-designed with only minor design flaws. Overall, although most sample sizes met requirements for statistical power, three out of five studies had sample sizes under 50 participants. Further, three out of five studies provided very limited baseline demographic and anthropometric subject data, which ultimately calls the generalizability of these studies into question. Lastly, most of the studies, with the exception of Lesperance et al. measured the magnitude of improvement of depressive symptoms, which provides insight not only into the statistical significance of the findings, but the clinical significance as well.

Probiotic Supplementation for Treatment of MDD

Evidence Summary

All five studies included in this evidence analysis utilized adult populations with either a formal diagnosis of MDD, as defined by the DSM-IV, or the presence of depressive symptoms confirmed by a validated tool, at screening. Overall, each study imposed relatively similar inclusion and exclusion criteria that controlled for serious comorbid medical conditions, pregnancy and lactation, psychotic disorders, and suicidal/homicidal ideation. All five studies used depressive symptom severity as the primary study outcomes, which were assessed using a variety of validated tools. Akkasheh et al. and Kazemi et al. elected to use the 21-item, self-reported Beck Depression Inventory (BDI), Pinto-Sanchez et al. utilized the self-reported Hospital Anxiety and Depression Scale (HAD), Romijn et al. employed the MADRS, iCGI, and QID-SR16, and Rudzki utilized the HAM-D. Intervention periods ranged from 6-8 weeks.

In total, the research fails to support the use of probiotic supplementation as monotherapy and provides conflicting evidence related to the effectiveness of combination therapy (with a SSRI or tricyclic antidepressant). Akkasheh et al. found that daily supplementation with approximately 6 billion CFU of a multispecies probiotic significantly improved depressive symptoms, although use of antidepressant medication was neither assessed nor controlled for in this study. Kazemi et al. also utilized a multispecies probiotic supplement (in a dose of ten billion CFU) in a sample undergoing concurrent therapy with either a SSRI or tricyclic antidepressant. Significant improvements in depressive symptoms were also observed in this intervention group compared to placebo. Pinto-Sanchez et al. found that supplementation with a single-

species probiotic (in a dose of 10 billion CFU) also yielded a significant improvement in depressive symptoms, although in this case, the sample population was not using antidepressant medications concurrently. Conversely, Romijn et al. and Rudzki et al. failed to find benefit from probiotic supplementation. In the case of Romijn et al., supplementation of approximately 3 billion CFU of a multispecies probiotic failed to yield beneficial effects in a population free of antidepressant medication. Rudzki et al. tested 20 billion CFU of a single-species probiotic (L. plantarum) on a population receiving either new or ongoing treatment with a SSRI and failed to observe favorable results.

Form and Dosage

Probiotic supplementation was highly heterogeneous amongst the five studies, which likely contributed to the inconsistent results that were observed. Those studies that observed improved depressive symptom severity in the intervention groups utilized probiotic supplements in doses of 6-10 billion CFU that contained either Bifidobacterium or a combination of Lactobacillus and Bifidobacterium. Those studies that failed to observe beneficial effects utilized supplements in doses of 3-20 billion CFU that contained either Lactobacillus or a combination of Lactobacillus and Bifidobacterium. An obviously superior form of administration was not observed across the studies, as capsules and powders were used in studies that observed beneficial effects as well as those that failed to observe favorable results. None of the studies disclosed whether the capsules used were enteric-coated.

Study Strengths and Limitations

All five studies were randomized controlled trials and were generally well-designed with only minor design flaws. Overall, although most sample sizes met

requirements for statistical power, four out of five studies had sample sizes under 80 participants. Interestingly, despite no apparent use of enteric-coated supplements, only one out of the five studies (Pinto-Sanchez et al.) elected to perform a fecal mircobiome analysis to measure treatment compliance and exposure. The studies did not employ consistent protocols in regards for controlling for probiotic consumption from supplement or dietary sources prior to, and during, the studies. Only Akkasheh et al. both excluded participants with recent use of probiotics and monitored dietary intake throughout the study.

Application to Practice

At present, the medical nutrition therapy for MDD is essentially limited to the promotion of a nutrient-dense diet to support overall well-being and to correct or prevent nutritional deficiencies. However, as previously discussed, patients with MDD often desire complementary or alternative treatments due to the high rate of side effects with antidepressant medications. At present, the research does not support incorporating either omega-3 fatty acids or probiotics as part of standardized treatment for MDD. However, given the demonstrated tolerability and safety of both omega-3 and probiotic supplements, dietetics practitioners can use clinical judgment to identify individual patients that may benefit from the addition of either omega-3 or probiotic supplements to be used concurrently with conventional treatment. In particular, patients with low-intake or poor acceptance/tolerance of dietary sources of omega-3 fatty acids, patients with comorbid IBS, or patients with metabolic or inflammatory conditions may benefit from supplementation. Current research appears to favor EPA-rich supplementation of at least 1100 mg/day and probiotic supplementation with a multispecies variety (that includes the genera Bifidobacterium) with at least 6 billion CFU per serving.

Recommendations for Future Research

Without question, a greater volume of research is required to develop more conclusive evidence to support or refute the use of omega-3 and probiotic supplements as either monotherapy or combination therapy for MDD. Based on the studies involved in this evidence analysis, additional 3-arm RCTs should be conducted to compare supplement monotherapy, combination therapy, and placebo. In general, larger sample sizes should be strived for in all future research. In order to confirm adequate bioavailability and appropriate supplement vehicle, all future research should assess baseline and study completion levels of serum omega-3 fatty acids (in studies investigating omega-3 supplements) and baseline and study completion fecal microbiome analysis (in studies investigating omega-3 supplements). In regard to research related to omega-3 supplements, future research should be designed to allow for up-titration of doses, particularly in studies that are investigating supplementation as monotherapy. Interestingly, while only one study in this evidence analysis utilized a total omega-3 dose greater than 2 grams, many recommendations regarding therapeutic uses of omega-3 fatty acids, such as those for preservation of lean body mass in cancer and for treatment of hypertriglyceridemia, recommend doses ranging from 2 to 4 grams per day. Given this, future research should involve similar doses for the treatment of MDD. In regard to research related to probiotic supplementation, studies that directly compare supplements with varying bacteria and CFU profiles would be highly informative. Finally, while a large body of evidence supports correlations between depression and inflammation, oxidative stress, immune dysfunction, and intestinal dysbiosis, review of the literature suggests that few studies adequately assess biochemical parameters that would give insight into the beneficial mechanisms of actions of omega-3 and probiotic

supplementation. Future research should consistently assess biomarkers such as CRP, IL-1β, IL-6, ESR, and TAC.

APPENDIX A

Table 2: Overview Table – Omega-3 Studies

Author, Year, Study Design, Class, Rating	Study Purpose	Study Population	Intervention	Outcomes	Limitations
Author: Gertsik et al. Year: 2012 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	Determine whether combined treatment with citalopram and omega-3 supplementation is more effective than antidepressant monotherapy. Secondly, to determine whether omega-3 supplementation leads to an accelerated antidepressant response.	42 participants with MDD and HAM-D score >17 Aged 18-65 (40.5 average age)	Eight weeks of treatment consisting of citalopram (initiated at 20 mg/d and increased up to 40 mg/d as needed at week 4) and omega-3 supplementation with two capsules twice daily containing 450 mg EPA, 100 mg DHA, and 50 mg other omega-3 fatty acids.	After controlling for citalopram dose, a significant improvement in HAM-D score over time was observed in the omega-3 group compared to the placebo group (p=0.008). Significant differences were noted at week 4, week 6, and at study termination. A significant improvement in remission status in the omega-3 group compared to the placebo group (p=0.018) was also observed.	Small sample size Questionable relevant population as very minimal subject demographics were provided. Study follow-up was 76%
Author: Jazayeri et al. Year: 2008 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To compare the therapeutic effects of EPA supplementation, fluoxetine monotherapy, and a combination of EPA supplementation plus fluoxetine therapy in patients with major depression.	48 participants (15 males, 33 females) with MDD and baseline HDRS score of ≥15 Free of antidepressant medication for ≥6 weeks Aged 20-59	Three treatment arms (each 8 weeks long): 1) Daily supplementation with two capsules of 550 mg ethyl-EPA 2) Daily 20 mg fluoxetine capsule 3) Daily supplementation with two capsules of 550 mg ethyl-EPA plus daily 20	Combination therapy with daily fluoxetine plus EPA supplementation was more effective at reducing depression symptoms than monotherapy with either fluoxetine or EPA supplementation (P=0.005 fluoxetine compared to combination; P=0.009 EPA compared to combination). There was no significant difference	Small sample size Intent-to-treat protocol was not used Placebo was not used Unclear if reduction in HDRS score was clinically significant

			mg fluoxetine capsule	in HDRS score at 8 weeks between the EPA and fluoxetine groups (P=0.426).	
Author: Lesperance et al. Year: 2011 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To determine whether EPA-rich omega-3 supplementation is more effective than placebo in reducing depressive sypmtoms over 8 weeks.	432 participants (136 males, 296 females) meeting diagnostic criteria for major depressive episode Mean age: 46.6 supplement group; 45.4 placebo group +/-current antidepressant use	Daily omega-3 fish oil supplementation (70% EPA, 5% DHA ethyl esters) providing the equivalent of 1050 mg/d EPA and 150 mg/d DHA for a total of 8 weeks.	After adjusting for confounding factors, a trend toward greater reduction of depressive symptoms in the EPA group compared to placebo was observed (P=0.088 for IDS-SR; P=0.053 for MADRS). EPA supplmentation conferred a greater benefit on those patients without comorbid anxiety.	P-values for baseline characteristics not provided. Sample was highly heterogeneous in regards to antidepressant use/type. Unclear if improvements observed in subjects without comorbid anxiety were clinically significant
Author: Mischoulon et al. Year: 2009 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To examine efficacy and safety of EPA supplementation as monotherapy in subjects with MDD.	41 (15 males, 26 females) with MDD and baseline HDRS score of ≥18 and CGI-S score of ≥3. Mean age of 42 No current antidepressant use	Daily supplementation with 1 g/d EPA for 8 weeks consisting of two capsules containing 500 mg of ethyl-ester EPA (plus 0.2% dl-α -tocopherol) of greater than 95% purity each.	In the completers sample (n=24), there was a trend for greater reduction of HDRS-17 score in the EPA group compared to control (P=0.087).	No comparison of subject characteristics was provided. Small sample size did not meet requirements for statistical power of 80%.

Author: Rudzki et al. Year: 2018 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To evaluate the effect of augmenting SSRI treatment with supplementation of Lactobacillus Plantarum 299v on cognitive, affective, and immune parameters of depressed patients .	79 (attrition: 17 male, 43 female) with MDD Mean age: 39.1 probiotic group, 38.9 placebo group +/-Current antidepressant (SSRI) treatment	SSRI treatment augmented with two capsules containing a total of 20 billion CFU of Lactobacillus plantarum 299v daily for 8 weeks.	There were no significant changes in endpoints related to affective symptoms in either group. Post hoc analysis demonstrated significant improvement in Work Speed in the AP Test ($P<0.001$), CVLT levels ($P=0.024$), KYN concentration ($P=0.017$), and 3HKYN:KYN ratio ($P=0.015$) in the probiotic group compared to placebo.	Unclear if improvements in cognitive performance were clinically significant

Table 3: Overview Table – Probiotic Studies

Author, Year, Study Design, Class, Rating	Study Purpose	Study Population	Intervention	Outcomes	Limitations
Author: Akkasheh et al. Year: 2016 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To assess whether probiotic supplementation favorably impacts depressive symptoms, glucose homeostasis, blood lipid profile, biomarkers of inflammation, and biomarkers of oxidative stress	40 participants (6 male, 34 female) with MDD and baseline HDRS score of ≥15 Age 20-55 (average: 36.2 placebo, 38.3 probiotic)	8 weeks of daily probiotic supplementation consisting of Lactobacillus acidophilus (2×10^9 CFU/g), Lactobacillus casei (2×10^9 CFU/g), and Bifidobacterium bifidum (2×10^9 CFU/g)	The probiotic group demonstrated significant improvements in BDI score (p=0.001), serum insulin (p=0.03), HOMA IR (p=0.03), hs-CRP (p=0.03), and GSH (p=0.02) compared to placebo. There was a trend towards significant improvements in HOMA-B (p = 0.06) and QUICKI (p = 0.07).	Small sample size Use of antidepressants not controlled for or discussed
Author: Kazemi et al. Year: 2018 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To compare the effect of probiotic and prebiotic supplementation vs placebo on decreasing depressive symptoms, kynurenine/tryptophan (KYN:TRP) ratio, and TRP/BCAAs ratio in adult subjects with mild to moderate MDD	110 participants (32 male, 78 female) with mild to moderate depression Age 18-50 (36.47 mean) Current use of antidepressant medication (tricyclic or SSRIs)	Daily supplementation with either 5g of an orally dispersible probiotic powder containing ten billion CFU of freeze-dried L. helveticus R0052 and B. longum R0175, or 5 g of an orally dispersible prebiotic powder containing galactooligosaccharide, for a total of 8 weeks	Mean BDI score significantly decreased in the probiotic group compared to the placebo and probiotic groups (P=0.042). After controlling for serum isoleucine, a significant decrease in KYN:TRP ratio was observed in the probiotic group compared to the placebo group (P=0.048).	Lack of response or remission rates gives little insight into clinical significance Lack of fecal microbiome analysis Heterogeneous treatment with antidepressant drugs Diagnostic criteria for depression unclear Unclear if comorbid psychiatric disorders were present

Author: Pinto-Sanchez et al. Year: 2017 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To evaluate the effect of B. longum supplementation on comorbid anxiety and depression, as well as on IBS symptoms, brain activation, inflammation, immune function, HPA axis function, microbiotia profile, and host-microbial metabolic interactions in individuals with IBS	44 participants (20 male, 24 female) with IBS and comorbid depression/anxiety with HAD scale score of 8-14 Age 26-58 No current use of antidepressant medication	Daily supplementation with probiotic powder containing 10 billion CFU of spray-dried B. longum NCC3001 for a total of 6 weeks	A significantly greater number of participants in the probiotic group experienced a reduction in HAD-D score of ≥2 points (P=0.04). Improvement in HAD-D scores in the probiotic group was sustained at 10 weeks. A sensitivity analysis revealed the beneficial effect of probiotic supplementation was more likely to occur in those participants who also reported adequate relief of IBS symptoms.	Lack of a clinician-administered rating of depression and anxiety (unclear how many subjects carried formal diagnosis of MDD) Unclear if participants were receiving psychotherapy during study Two study authors were employees of Nestec SA, two authors were employess of Nestle Institute of Health Sciences SA
Author: Romijn et al. Year: 2017 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To test whether probiotic supplementation improved depression symptoms in individuals with low mood, and to examine whether the presence or severity of IBS symptoms and levels of inflammatory biomarkers would predict or impact treatment response.	79 participants (43 male, 36 female) with either score of ≥11 on the QIDS-SR16 or ≥14 on the depression subscale of the DASS-42 Mean age: 35.8 probiotic group, 35.1 placebo group Free from antidepressant medication for at least 4 weeks	Daily supplementation with at least three billion CFU of freeze-dried L. helveticus R0052 and B. longum R0175 per 1.5 g sachet of orally dispersible powder for a total of 8 weeks	No significant group differences were observed for any outcome measure following both initial and secondary analyses. Exploratory analyses revealed that individuals in the probiotic group with high baseline vitamin D levels showed greater improvement in psychological outcomes than those who had low baseline vitamin D levels (P<0.05 for iCGI, QIDS-SR16, and GAF individually).	Dietary sources of probiotics were not measured or controlled for Potentially relevant anthropometric variables were not measured (such as BMI, body fat percentage) Fecal microbiome analysis was not completed to measure exposure or patient compliance

Author: Rudzki et al. Year: 2018 Study Design: Randomized Controlled Trial Class: A Rating: Positive (+)	To evaluate the effect of augmenting SSRI treatment with supplementation of Lactobacillus Plantarum 299v on cognitive, affective, and immune parameters of depressed patients .	79 (attrition: 17 male, 43 female) with MDD Mean age: 39.1 probiotic group, 38.9 placebo group +/-Current antidepressant (SSRI) treatment	Daily supplementation with two capsules of 10 billion CFU of Lactobacillus plantarum 299v for a total of 8 weeks.	There were no significant changes in endpoints related to affective symptoms in either group. Post hoc analysis demonstrated significant improvement in Work Speed in the AP Test ($P<0.001$), CVLT levels ($P=0.024$), KYN concentration ($P=0.017$), and 3HKYN:KYN ratio ($P=0.015$) in the probiotic group compared to placebo.	Unclear if improvements in cognitive performance were clinically significant

APPENDIX B

Table 4: Quality Criteria Summary – Omega-3 Studies

Quality Criteria Summary					
	Gertsik et al., 2012	Jazayeri et al., 2008	Lesperance et al., 2011	Mischoulon et al., 2009	Mischoulon et al., 2015
Relevance Questions					
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group?	Yes	Yes	Yes	Yes	Yes
2. Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes	Yes	Yes	Yes	Yes
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	Yes	Yes	Yes	Yes	Yes
4. Is the intervention or procedure feasible?	Yes	Yes	Yes	Yes	Yes
Validity Questions					
1. Was the research question clearly stated?	Yes	Yes	Yes	Yes	Yes
2. Was the selection of study subjects/patients free from bias?	Yes	Yes	Yes	Yes	Yes
3. Were the study groups comparable?	Yes	Yes	Yes	Yes	Yes
4. Was method of handling withdrawals described?	Yes	Yes	Yes	Yes	Yes
5. Was blinding used to prevent introduction of bias?	Unclear	Yes	Yes	Yes	Yes
6. Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?	Yes	Yes	Yes	Yes	Yes
7. Were outcomes clearly defined and the measurements valid and reliable?	Yes	Yes	Yes	Yes	Yes
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?	Yes	Yes	Yes	Yes	Yes
9. Are conclusions supported by results with biases and limitations taken into consideration?	Yes	Yes	Yes	Yes	Yes
10. Is bias due to study's funding or sponsorship unlikely?	Yes	Yes	Yes	Yes	Yes

Table 5: Quality Criteria Summary – Probiotics Studies

Quality Criteria Summary					
	Akkasheh et al., 2016	Kazemi et al., 2018	Pinto-Sanchez et al., 2017	Romijn et al., 2016	Rudzki et al., 2018
Relevance Questions					
1. Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group?	Yes	Yes	Yes	Yes	Yes
2. Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes	Yes	Yes	Yes	Yes
3. Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to dietetics practice?	Yes	Yes	Yes	Yes	Yes
4. Is the intervention or procedure feasible?	Yes	Yes	Yes	Yes	Yes
Validity Questions					
1. Was the research question clearly stated?	Yes	Yes	Yes	Yes	Yes
2. Was the selection of study subjects/patients free from bias?	Yes	Yes	Yes	Yes	Yes
3. Were the study groups comparable?	Yes	Yes	Yes	Yes	Yes
4. Was method of handling withdrawals described?	Yes	Yes	Yes	Yes	Yes
5. Was blinding used to prevent introduction of bias?	Yes	Yes	Yes	Yes	Yes
6. Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?	Yes	Yes	Yes	Yes	Yes
7. Were outcomes clearly defined and the measurements valid and reliable?	Yes	Yes	Yes	Yes	Yes
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?	Yes	Yes	Yes	Yes	No
9. Are conclusions supported by results with biases and limitations taken into consideration?	Yes	Yes	Yes	Yes	Yes
10. Is bias due to study's funding or sponsorship unlikely?	Yes	Unclear	Unclear	Yes	Yes

APPENDIX C

Citation	Gertsik L, Poland RE, Bresee C, Hyman Rapaport M. Omega-3 fatty acid augmentation of Citalopram treatment for patients with major depressive disorder. J Clin Psychopharmacol. 2012;32(1):61-64. doi:10.1097/JCP.0b013e31823f3b5f
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	+ (positive)
Research Purpose	Determine whether combined treatment with citalopram and omega-3 supplementation is more effective than antidepressant monotherapy. Secondly, to determine whether omega-3 supplementation leads to an accelerated antidepressant response.
Inclusion Criteria	• 18-65 years of age • meet DSM-IV criteria for major depressive disorder by Structured Clinical Interview for DSM Disorders and have a Hamilton Depression Rating Scale (HAM-D) score >17 • women of childbearing age using effective contraception
Exclusion Criteria	• diagnosis of psychotic disorders • current drug or alcohol disorders • unstable medical or neurological disorders • history of allergy to citalopram, omega-3 fatty acids, finfish or shellfish • history of failure to respond to citalopram • history of seizure disorder • pregnancy • need for concomitant therapy of other psychotropic medications • active suicidal ideation or other safety concerns • exposure to fluoxetine or MAOIs within the past 2 months • current anticoagulant therapy • dietary intake >3.0g total omega-3 per day

Description of Study Protocol	**Recruitment:** local advertisement; physician referral **Design:** Participants were enrolled in a one week, single-blind, placebo run-in phase. Qualified subjects were then block randomized by gender into control and treatment groups. Both groups received citalopram (20 mg/day initially) for eight weeks. The control group was instructed to take 1000 mg capsule of olive oil twice daily with meals for eight weeks. The treatment group was instructed to take two omega-3 capsules twice daily with meals for eight weeks. Citalopram was increased to 40 mg/day at week 4 if HAM-D scores had decreased <25%. Participants were assessed by a study psychiatrist at baseline, randomization, and study weeks 2, 4, 6, and 8. Plasma C-reactive protein and 24-hour urinary cortisol levels were collected at baseline and study termination. **Blinding used:** single-blind **Intervention:** Eight weeks of treatment consisting of citalopram (initiated at 20 mg/d and increased up to 40 mg/d as needed at week 4) and omega-3 supplementation with two capsules twice daily containing 450 mg EPA, 100 mg DHA, and 50 mg other omega-3 fatty acids. **Statistical Analysis:** Prior to analysis, Kolmogorov-Smirnov test was used to test continuous data for normal distribution. Demographic, psychiatric and medical characteristics, differences in citalopram dosing, protocol completion, and adverse events/effects were compared using both parametric (Chi-square, Student's t-test) and non-parametric (Fisher Exact Tests, Wilcoxon rank sum tests) were used when appropriate. Intent-to-treat was applied for analysis for efficacy. A mixed-effects linear regression model with ante-dependence covariance structure was used to test the primary hypothesis. Changes in citalopram dosing was controlled for. Subjects were classified as having full remission, partial remission, partial improvement, and no improvement based on HAM-D scores. Distribution of the number of these subjects for each classification was compared across treatment groups with a Wilcoxon Rank Sum Test. Secondary measures (changes in BDI and MARDRS scores) were analyzed with mixed model linear regression. 18-26 subjects were needed per group for 80% power.
Data Collection Summary	**Timing of Measurements:** HAM-D, BDI, MADRS, CGI, and PGI were assessed at baseline, randomization, and study weeks 2, 4, 6, and 8. Plasma c-reactive protein and 24-hour urinary cortisol were measured at baseline and study termination. **Dependent Variables:** Primary: HAM-D score; Secondary: BDI, MADRS, CGI, PGI, CRP, 24-urinary cortisol **Independent Variables:** Two omega-3 capsules twice daily (each capsule contained 450 mg EPA, 100 mg DHA, and 50 mg other omega-3 fatty acids). **Control variables:** Initial citalopram dosing of 20 mg/day (taken in the morning).

Description of Actual Data Sample	**Initial:** 42 **Attrition:** 32 **Age:** 40.5 (average) **Ethnicity:** not provided **Other Relevant Demographics:** Baseline subject characteristics were not provided in table format. The following comment was provided in the results section: "baseline demographic, medical and depression history measures were similar between the two study groups. The only significant difference between the two study groups at baseline was a higher MADRS score in the placebo-treated group…" **Anthropometrics:** not provided **Location:** Los Angeles, CA
Summary of Results	**Key Findings:** After controlling for citalopram dose, there was a significant improvement in HAM-D score over time in the omega-3 treated group compared to the placebo-treated group (p=0.008). Significant differences were noted at week 4, week 6, and at study termination. There was a significant improvement in remission status in the omega-3 group compared to the placebo group (p=0.018). **Other Findings:** No significant changes in CRP or 24-hour urinary cortisol levels were observed.
Author Conclusion	Initiation of treatment with an SSRI (citalopram) augmented with daily omega-3 supplementation is more effective at reducing depressive symptoms when compared with SSRI monotherapy.
Reviewer Comments	• *Study follow-up was 76%.* • *Small sample size* • *Minimal sample characteristics provided*
Funding Source	Provided by National Institute of Health's National Center for Complementary and Alternative Medicine and the National Center for Research Resources.

Citation	Jazayeri S, Tehrani-Doost M, Keshavarz SA, et al. Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine, separately and in combination, in major depressive disorder. Aust N Z J Psychiatry.2008;42:192-198.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	To compare the therapeutic effects of EPA supplementation, fluoxetine monotherapy, and a combination of EPA supplementation plus fluoxetine therapy in patients with major depression.
Inclusion Criteria	• age 20-59 • met DSM-IV criteria for MDD without psychotic features based on the semi-structured clinical interview • score of >/=15 on the HDRS • free of medication for at least 6 weeks
Exclusion Criteria	• comorbid psychiatric diagnosis other than dysthymia and anxiety • significant medical illness established by medical history, physical examination, or laboratory tests • suicidal thoughts • substance abuse • history of hypomanic/manic/mixed episode • pregnancy and lactation • consumption of omega-3 supplements in the previous year • dietary intake of more than one serving of fish per week
Description of Study Protocol	**Recruitment:** Participants were recruited from Roozbeh Psychiatry Hospital. **Design:** Participants were assigned to three groups according to pre-arranged balanced block randomization to receive either two daily capsules of EPA (1000 mg total) plus fluoxetine placebo, or one capsule of fluoxetine (20 mg total) with EPA placebo, or two capsules of EPA (1000 mg total) plus fluoxetine (20 mg total) for a total of 8 weeks. **Blinding used:** double blind (with double dummy technique) **Intervention:** Three treatment arms (each 8 weeks long): daily supplementation with two soft gel capsules of 550 mg ethyl-EPA (500 mg pure EPA and 11 mg vitamin E) with placebo fluoxetine capsule; daily 20 mg fluoxetine capsule with two placebo EPA capsules; daily EPA (two 550 mg ethyl-EPA capsules) + daily fluoxetine capsule (20 mg)

	Statistical Analysis: ANCOVA was used to compared HDRS score at 8 weeks across treatment groups with baseline HDRS, age of depression onset, and number of previous episodes as covariates. Repeated measure ANOVA was used to analyze differences in HDRS scores over time within each group. Differences were considered significant for $P<0.05$.
Data Collection Summary	**Timing of Measurements:** HDRS score was clinician-measured at baseline and at weeks 2, 4, 6, and 8. **Dependent Variables:** Change in depressive symptoms as measured by HDRS score. **Independent Variables:** EPA intake, fluoxetine intake, and EPA + fluoxetine intake
Description of Actual Data Sample	**Initial:** 60 **Attrition:** 48 (15 males, 33 females) **Age (average):** 35.1 fluoxetine group; 34.9 EPA group; 34.5 fluoxetine+EPA group **Ethnicity:** Iranian **Other Relevant Demographics:** • Number of previous depressive episodes: 0.38 fluoxetine, 0.5 EPA, 1.38 fluoxetine+EPA (P=0.26) • age of onset: 32.9 fluoxetine, 31.4 EPA, 28.1 fluoxetine+EPA (P=0.29) • duration of recent episode (weeks): 13.3 fluoxetine, 12.6 EPA, 12.9 fluoxetine+EPA (P=0.98) • baseline HDRS score: 29.31 fluoxetine, 29.94 EPA, 31.63 fluoxetine+EPA (P=0.50) **Anthropometrics:** Weight: 73.63 fluoxetine, 67.86 EPA, 65.83 fluoxetine+EPA (P=0.32) **Location:** Tehran, Iran
Summary of Results	**Key Findings:** Combination therapy with daily fluoxetine plus EPA supplementation was more effective at reducing depression symptoms than monotherapy with either fluoxetine or EPA supplementation (P=0.005 fluoxetine compared to combination; P=0.009 EPA compared to combination). There was no significant difference in HDRS score at 8 weeks between the EPA and fluoxetine groups (P=0.426). **Other Findings:** Treatment (P=0.005), age of onset (P=0.009), and baseline HDRS score (P=0.027) had a significant effect on HDRS score at week 8. ANCOVA for HDRS score at weeks 2, 4, and 6 revealed that treatment had an effect on HDRS score at week 4 and 6 (P=0.016, 0.02 respectively). Response rates, as indicated by a reduction in baseline HDRS score of >/=50%, were 50% in the fluoxetine group, 56% in the EPA group, and 81% in the combination group. Within-group analysis revealed a significant effect of time within each group and when all groups were combined, with a noticeable effect starting at week 2

	(P<0.05).
Author Conclusion	Daily EPA supplementation is as effective as fluoxetine as monotherapy for MDD. EPA used in combination with fluoxetine is superior in reducing depressive symptoms compared to monotherapy with fluoxetine or EPA.
Reviewer Comments	• *Does not appear that ITT protocol was used.* • *Small sample size* • *Placebo not used. As a result, cannot confirm whether EPA supplementation or fluoxetine is a more effective therapy than a placebo.* • *Unclear if improvement in HDRS score was clinically significant. P-value for response rates between groups was not provided*
Funding Source	Provided by Tehran University of Medical Sciences.

Citation	Lesperance F, Frasure-Smith N, St-Andre E, Turecki G, Lesperance P, Wisniewski SR. The efficacy of omega-3 supplementation for major depression: A randomized controlled trial. J Clin Psychiatry. 2011;72(8:1054-1062.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	To determine whether EPA-rich omega-3 supplementation is more effective than placebo in reducing depressive sypmtoms over 8 weeks.
Inclusion Criteria	• ≥18 years old • meet diagnostic criteria for major depressive episode (MDE) based on the Mini-International Neuropsychiatric Interview (MINI) • baseline score of ≥27 on the self-report Inventory of Depressive Symptomatology • presence of clinically significant depressive symptoms for ≥4 weeks • if taking antidepressants, to have been at maximim tolerated dosage for >4 weeks • if not on antidepressants, to have been intolerant to ≥2 previous antidepressants or to refuse to take antidepressants despite medical advice • to have signed an informed consent
Exclusion Criteria	• known allergy to fish or sunflower oil • history of fish intolerance • having taken >14 g of omega-3 supplements during the past 4 weeks • diagnosis of drug or alcohol abuse or dependency during the past 12 months • presence of bipolar disorder based on the MINI • significant suicidal risk based on clinical judgement • history of myocardial infarction • history of pancreatic insufficiency • history of coagulation diseases or regularly taking drugs/herbs with antiplatelet or anticoagulant properties • nonmenopausal women with positive pregnancy tests or not using an accepted method of contraception

Description of Study Protocol	**Recruitment:** advertisements in medical centers and mass media; physician referrals; from caseloads of study investigators **Design:** Randomization was stratified by study site and baseline antidepressant use/nonuse. At baseline evaluation, the study psychiatrist administered the MINI, reviewed participants' medical and psychiatric history, and obtained written informed consent. Participants completed the self-report IDS-SR and the study clinicians administered the Montgomery-Asberg Depression Rating Scale (MADRS). Patients returned for clinic visits at weeks 1, 2, 4, and 8 for ongoing outcome assessment, monitoring of progress, and screening for adverse events. Participants were instructed to take 3 capsules of either omega-3 fish oil or sunflower oil placebo (with added 2% fish oil to support blinding) daily. Participants were asked to not change their usual dietary intake. **Blinding used:** double-blind (integrity of double-blind was evaluated at the end of week 1; participants' responses permitted calculation of the James' blinding index) **Intervention:** Daily omega-3 fish oil supplementation (70% EPA, 5% DHA ethyl esters) providing the equivalent of 1050 mg/d EPA and 150 mg/d DHA. **Statistical Analysis:** Analysis was performed on an intent-to-treat basis, with 2-sided tests. P≤0.49 was used to define statistical significance for the primary outcome and P≤0.05 was used for all other analyses. It was ultimately determined that a sample of 432 would provide 80% power. Mixed-effect regression models were used to test group differences in both the primary and secondary outcomes, with baseline scores as covariates. Mixed-effect regression models were used to assess the potential effects of antidepressent treatment at baseline, comorbid anxiety disorders, sex, and dietary intake of fatty fish for one month before baseline.
Data Collection Summary	**Timing of Measurements:** Both the primary outcome (IDS-SR) and secondary outcome (MADRS) were measured at baseline and at weeks 1, 2, 4, and 8. **Dependent Variables:** Change in depression symptoms as measured by change in IDS-SR and MADRS scores. **Independent Variables:** omega-3 fatty acid intake (via daily supplementation) **Control variables:** dietary omega-3 fatty acid intake
Description of Actual Data Sample	**Initial:** 432 (136 males, 296 females) **Attrition:** 361 **Age (mean):** 46.6 supplement group, 45.4 placebo group **Ethnicity:** not provided **Other Relevant Demographics:** • Current smoker: 20.2% supplement group, 16.9% placebo

	group • Use of omega-3 supplement in the preceding month: 11.9% supplement group; 14% placebo group • 2 or more portions of fish/week in the preceding month: 29.8% supplement group, 35% placebo group • Baseline antidepressant use: 41.7% supplement group, 38.8% placebo group • Baseline psychotherapy: 17.4% supplement group, 12.1% placebo group • Recurrent depression: 71.6% supplement group; 73.4% placebo group • >2 year duration of depression: 29.2% supplement group, 24.8% placebo group • Comorbid anxiety disorder: 52.8% supplement group, 52.8% placebo group **Anthropometrics:** • Obesity: 27.8% supplement group, 24.9% placebo group **Location:** Canada
Summary of Results	**Key Findings:** After adjusting for baseline score, week of assessment, adjuvant antidepressant use at baseline, and study site, there were insignificant trends toward superiority of EPA supplementation in reducing mean IDS-SR (P=0.088) and MADRS (P=0.053) scores, with small effect sizes (IDS-SR=0.11; MADRS=0.10). The interaction of comorbid anxiety disorders and study group was significant (P=0.035). Patients without comorbid anxiety benefited from EPA supplementation, in which the mean adjusted difference over the trial between patients taking EPA supplements and those taking the placebo was 3.17 points on the IDS-SR (P=0.007; effect size of 0.27) and 1.93 points on the MADRS (P=0.008; effect size of 0.26). **Other Findings:** There was no evidence of interactions for use of antidepressants at baseline (P=0.33), gender (P=0.78), or servings of fish consumed per week in the month before the study (P=0.18). Fishy aftertaste was the only nonserious adverse event that was observed to be significantly more frequent in the supplement group compared to placebo group.
Author Conclusion	This study does not provide sufficient evidence of the efficacy of EPA-rich omega-3 supplementation in reducing depressive symptoms compared to placebo. However, omega-3 supplementation was superior to placebo in reducing depressive symptoms in those patients without co-morbid anxiety compared to placebo.
Reviewer Comments	• *P-values for participants' baseline characteristics were not provided. The only reference to the similarity of the two groups was in the results section: "the groups were well balanced."* • *The sample in this study was more heterogeneous than those samples in phase 3 antidepressant trials.* • *Unclear if positive results were clinically signficant -- is the reduction in the IDS-SR and MADRS score clinically meaningful in regards to treatment efficacy? Authors did not identify a target treatment response.*

Funding Source	Provided by an unrestricted grant from Isodis Natura (who also provided study medication and placebo), the Fondation du Centre Hospitalier de l'Universite de Montreal, and the CRCHUM.

Citation	Mischoulon D, Papakostas GI, Dording CM, et al. A double-blind, randomized controlled trial of ethyl-eicosapentaenoate for major depressive disorder. J Clin Psychiatry. 2009;70(12):1636-1644.doi:10.4088/JCP.08m04603.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	To examine efficacy and safety of EPA supplementation as monotherapy in subjects with MDD. Additionally, to examine the relationship between severity of depression and baseline plasma levels of EPA, DHA, n-6/n-3 ratio, and the impact of EPA supplementation on these parameters. Finally, to examine the impact of consumption of omega-3-rich foods on depression severity and response to EPA supplementation.
Inclusion Criteria	• meet criteria for MDD as set out in the Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition (SCID-I/P) • ability to provide written, IRB-approved informed consent • aged 18-80 years • baseline HDRS score of ≥18 • baseline Clinical Global Impression Severity of Illness Scale score of ≥3
Exclusion Criteria	• pregnancy or no use of medically accepted means of contraception in women of child-bearing potential • breastfeeding • current, serious suicidal or homicidal risk • serious or unstable medical illness (including cardiovascular, hepatic, renal, respiratory, endocrine, neurologic, or hematologic diseases) • history of unstable seizure disorder • use of anticoagulants • DSM-IV diagnoses including organic mental disorders, substance abuse disorders including alcohol (active within the last 6 months), schizophrenia, delusional disorder, bipolar disorder • history of multiple adverse drug reactions or allergy to the study drugs • psychotic features • current use of antidepressants, lithium, or anticonvulsants for mood stabilization • evidence of hypothyroidism • current use of other psychotropic drugs • failure to respond during the course of current major depressive episode to at least one antidepressant trial • having taken at least 1g/d of an omega-3 product, or any current supplement enriched with omega-3 fatty acids • history of electroconvulsive therapy within 6 months

Description of Study Protocol	**Recruitment:** advertisements and referrals to the MGH Depression Clinical and Research Program **Design:** Subjects were randomly assigned to either receive 2 capsules of EPA or placebo (containing paraffin oil and 0.2% dl-alpha-tocopherol) for 8 weeks. Capsules were to be taken twice daily or together at the same time. Subjects were seen at screening, baseline, and then every 2 weeks for a total of 8 weeks. Participants were asked to complete daily food diaries (Mallinckrodt General Clinical and Research Center Food Record). Subjects were encouraged not to modify their regular diet during the study. **Blinding used:** double blind **Intervention:** Daily supplementation with 1 g/d EPA for 8 weeks consisting of two capsules containing 500 mg of ethyl-ester EPA (plus 0.2% dl-α -tocopherol) of greater than 95% purity each. **Statistical Analysis:** Completer and intent-to-treat analyses of participants with at least 1 post-baseline evaluation visit were performed. Chi-square and Fisher exact tests were used to compare response and remission rates, and the difference in dropout rates between the two groups. Mann-Whitney U test was used to compare the degree of clinical improvement between groups. ANOVA and Mann-Whitney U test were used to compare degrees of improvement between subjects consuming different levels of dietary omega-3, and to assess changes in plasma lipid parameters. Linear regression was used to assess the relationship between plasma lipids and baseline depression severity and improvement in HDRS-17 score. Logistic regression was used to assess the relationship between baseline plasma lipids and treatment response, and the associations between degree of change in plasma lipids, improvement in HDRS-17 score, and treatment. Mann-Whitney U test was used to assess the relationship between dietary omega-3 intake, baseline plasma lipids, and treatment response. 2-sided significance was set at $P<0.05$ for all analyses.
Data Collection Summary	**Timing of Measurements:** The primary outcome (HDRS-17 score) was measured at baseline and every 2 weeks for a total of 8 weeks. Plasma levels of EPA, DHA, total omega-3, total omega-6, and omega-6/omega-3 ratio were measured at baseline and 8 weeks. **Dependent Variables:** Change in HDRS-17 score (with clinical response defined as a 50% or greater decrease and remission defined as final HDRS-17 score of 7 or less); plasma lipid levels (EPA, DHA, total omega-3, total omega-6, and omega-6/omega-3 ratio). **Independent Variables:** EPA intake (via daily supplementation) **Control variables:**

Description of Actual Data Sample	**Initial:** 41 (15 males, 26 females) **Attrition:** 35 **Age (mean):** 42 **Ethnicity:** not provided **Other Relevant Demographics:** not provided **Anthropometrics:** not provided **Location:** Massachusetts, US
Summary of Results	**Key Findings:** In the completers sample (n=24), there was a nonsignificant trend for greater reduction of HDRS-17 score in the EPA group compared to control (P=0.087). There was not a significant difference in response rate or remission rates between the two groups. In the ITT sample, there was no significant difference in reduction of HDRS-17 score, response rate, or remission rate between the two groups. **Other Findings:** For all subjects, no significant associations between any of the baseline plasma lipid parameters and depression severity were found. For EPA completers, a significant correlation between baseline omega-6/omega-3 ratio and change in HDRS-17 score with treatment was observed (P=0.030). For placebo completers, a significant correlation between baseline DHA levels and change in HDRS-17 score (P=0.033), and between baseline total omega-3 levels and change in HDRS-17 score (P=0.032) were observed. For EPA subjects, a significant correlation between change in omega-6/omega-3 ratio and change in HDRS-17 score was observed (P=0.032). In the ITT EPA sample, a significant association between baseline omega-6/omega-3 ratio and treatment response (P=0.032) was observed. Among EPA subjects in the ITT group, those with low dietary omega-3 consumption had the most robust response rate of 33% with progressively lower response rates in the medium (25%), high (0%), and pooled/"adequate" (20%) intake groups.
Author Conclusion	EPA supplementation provided a nonsignificant advantage over placebo in reducing depressive symptoms but was not superior in improving response or remission rates compared to placebo. This study does not provide evidence for use of EPA supplementation as a first-line monotherapy.
Reviewer Comments	• *No comparison of subject characteristics was provided.* • *The study had limited statistical power as a sample size of 80 subjects was required for a power of 80%.*
Funding Source	Funded by a grant from the National Center for Complementary and Alternative Medicine, National Institute of Health.

Citation	Mischoulon D, Nierenberg AA, Schettler PJ, et al. A double-blind, randomized controlled clinical trial comparing eicosapentaenoic acid versus docosahexaenoic acid for depression. J Clin Psychiatry. 2015;76(1)54-61.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	To compare two omega-3 preparations (EPA- vs. DHA-rich) as monotherapy for unmedicated adults with MDD.
Inclusion Criteria	• diagnosis of MDD per Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Edition (SCID I/P) • Clinical Global Impressions-Severity of Illness scale (CGI-S) score ≥3 • baseline 17-item Hamilton Depression Rating Scale (HDRS-17) score ≥15
Exclusion Criteria	• mean dietary omega-3 intake of ≥3.0 g/day • pregnancy or women of childbearing potential who were not using medically accepted means of contraception • suicidality or homicidality • serious or unstable medical illness • current or past history of organic mental disorders, substance abuse disorders, any psychotic disorders, and bipolar disorder • history of multiple adverse drug reactions or allergy to the study compounds • concurrent use of psychotropic medications, systematic corticosteroid or steroid antagonists, anticoagulants, or immunosuppressant agents • electroconvulsive therapy during the current episode • any trial of ≥6 weeks with citalopram 40 mg/d or equivalent antidepressant during the current episode • history of use of 1 g/d of omega-3 supplements • history of bleeding disorder • psychotherapy • smoking >10 cigarettes per day • vitamin E supplementation >400 IU • menstruating individuals unable to have baseline and posttreatment blood drawn during the follicular phase • individuals unable to refrain from nonsteroidal anti-inflammatory use for >72 hours prior to bloodwork • subjects with CGI-I score of 1 or 2 during baseline visit

Description of Study Protocol	**Recruitment:** advertisements and referrals from outpatient programs **Design:** Participants were randomized to receive either 1000 mg/d of EPA-enriched supplement or 1000 mg/d DHA-enriched supplement for 8 weeks. Participants took two EPA capsules plus two identical placebo capsules, or 4 DHA-rich capsules, or 4 placebo capsules, every morning. A double-dummy placebo design was used as the DHA capsules differed in appearance from the EPA capsules. Participants were asked not to significantly modify their diet during the study. Compliance was determined by pill count from bottles returned at follow-up visits. **Blinding used:** double-blind (with double-dummy placebo design) **Intervention:** Daily supplementation with either 1000 mg/d of EPA-enriched omega-3 (EPA:DHA=4:1) or 1000 mg/d of DHA-enriched omega-3 (DHA:EPA=5:1) for 8 weeks. **Statistical Analysis:** Analysis was performed based on a modified intent-to-treat basis. Comparisons across groups at baseline were made using ANOVA (continuous variables) and x^2 tests (categorical variables). Mixed-model repeated measures analysis was used to examine treatment group effect on changes from baseline to week 8 for depression severity, well-being, and quality of life (subjects were treated as random effect and treatment group and study week were treated as fixed effects). An auto-regressive covariance structure was used. Site and baseline scores were used covariates in all models. Treatment response was defined as improvement in HDRS-17 of ≥50% from baseline to week 8. Remission was defined as final HDRS-17 score of ≥7. Comparisons in response and remission rates, as well as CGI-S and CGI-I at week 8 were made using x^2 analysis. Comparison of adverse events was compared between treatment groups using ANOVA and x^2 tests.
Data Collection Summary	**Timing of Measurements:** HDRS-17 score (primary outcome), CGI-S score, CGI-I score, 16-item Quick Inventory of Depressive Symptomatology-Self-Report (QIDS-SR-16) score, Well-Being Scale (WBS) score, and Quality of Life Satisfactions Questionnaire (Q-LES-Q) score were measured every 2 weeks for a total of 8 weeks. **Dependent Variables:** Depressive symptoms, quality of life, and well-being as measured by: HDRS-17, CGI-S, CGI-I, QIDS-SR-16, WBS, Q-LES-Q. **Independent Variables:** omega-3 supplementation (EPA- or DHA-enriched) **Control variables:** omega-3 dietary intake

Description of Actual Data Sample	**Initial:** 196
	
	Attrition: 177 (72 males, 105 females)
	
	Age: 21-73 (45.8 mean)
	
	Ethnicity:
	• Hispanic (15.8%)
	• Non-hispanic (84.2%)
	• Race:
	  o Caucasian (67.8%)
	  o African American (18.1%)
	  o Other (8.5%)
	  o Prefer not to say (5.6%)
	
	Other Relevant Demographics:
	• Education:
	  o high school or less (26%)
	  o some college or more (74%)
	• Marital status:
	  o married/cohabitating (19.5%)
	  o separated/widowed/divorced (33.3%)
	  o never married (47.2%)
	• Employment status (P=0.041):
	  o employed (47.6%)
	  o homemaker (4.7%)
	  o student (6.5%)
	  o other (41.2%)
	
	Anthropometrics: not provided
	
	Location: Massachusetts, US

Summary of Results	**Key Findings:** All 3 groups experienced significant improvement in HDRS-17, QIDS-SR-16, CGI-S, Q-LES-Q, and WBS scores. Effect sizes between group pairs did not suggest a meaningful advantage for any treatment. There was no significant difference in response and remission rates between groups. **Other Findings:** The only significant difference in baseline characteristics between the 3 groups was employment status (P=0.041) in which the DHA group had significantly more employed participants. 87% of 177 evaluable subjects completed the study. For each treatment arm, response rates were between 40-50% and remission rates were ~30%. Those with comorbid anxiety disorder had a medium treatment effect size for HDRS-17 improvement by week 8 (-0.43 and -0.47 for EPA- and DHA-enriched supplements, respectively, vs placebo, P=.489). In regards to tolerability, 2 symptoms were significantly different by treatment group: constipation (13.3% for EPA, 14.3% for DHA, and 0% for placebo, P=0.010) and tremors (1.7% for EPA, 8.9% for DHA, and 0% for placebo, P=0.020). Subjects with greater depressive severity had a more robust improvement with EPA-enriched treatment.

Author Conclusion	Neither EPA-enriched nor DHA-enriched omega-3 supplementation was more effective than placebo as monotherapy for depression.
Reviewer Comments	• *Original power estimates required 100 subject for each treatment arm for a power of 80% (to detect an effect size >0.40).* • *3-arm design may have contributed to high placebo response rate (47.5%), which may have been influenced by subject expectancy and enthusiasm, and benign treatment side effect profiles.*
Funding Source	Funded by grant 5R01MH740585 from the National Institutes of Health.

Citation	Akkasheh, G, Kashani-Poor, Z, Tajabadi-Ebrahimi, M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. Nutrition. 2016;32: 315-320.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	To assess whether probiotic supplementation favorably impacts depressive symptoms, glucose homeostasis, blood lipid profile, biomarkers of inflammation, and biomarkers of oxidative stress.
Inclusion Criteria	• Age 20-55 • diagnosis of major depressive disorder as defined by the DSM-IV • Hamilton Depression Rating Scale score of ≥15
Exclusion Criteria	• history of coronary infarction or angina pectoris • current pregnancy or lactation • substance abuse • use of dietary or probiotic supplementation use within the past 2 months
Description of Study Protocol	**Recruitment:** Subjects were referred from Kargarneghad Hospital. **Design:** Participants were randomly allocated to two groups to receive either a probiotic supplement or a placebo for 8 weeks. Participants in the probiotic group were instructed to take one probiotic capsule daily. Participants in the placebo group were similarly instructed to take one capsule daily, which was identical in appearance to the probiotic supplement. Participants were instructed to not alter their usual dietary intake or physical activity for the duration of the study. Compliance to the probiotic and placebo products was assessed by asking participants to return the product containers. Three-day dietary and physical activity records were collected at week 2, 4, and 6 to ensure that participants maintained their usual routines. Body weight and height were measured by a trained nutritionist at recruitment and study termination. **Blinding used:** double-blind **Intervention:** 8 weeks of daily probiotic supplementation consisting of Lactobacillus acidophilus (2×10^9 CFU/g), Lactobacillus casei (2×10^9 CFU/g), and Bifidobacterium bifidum (2×10^9 CFU/g). **Statistical Analysis:** Kolmogorov-Smirnov test was used to determine the normal distribution of variables. Analysis was conducted based on an intent-to-treat basis. The independent samples student's t test was used to detect differences in variables/characteristics between the two groups. One-way repeated

	measure analysis was used to analyze intervention effect on biomarker outcomes. Within-group analysis was conducted using the paired-samples t test. Analysis of covariance was used to control for confounders. A P-value of <0.05 was considered statistically significant. 34 subjects were needed for 80% power.
Data Collection Summary	**Timing of Measurements:** Beck Depression Inventory (BDI) score was measured at baseline and study termination. Fasting blood samples of the following biomarkers were obtained at baseline and study termination: fasting plasma glucose (FPG), insulin, HOMA-B, HOMA-IR, QUICKI, triglycerides, VLDL-C, LDL-C, HDL-C, total cholesterol, hs-CRP, TAC, and GSH. **Dependent Variables:** Primary: BDI score; Secondary: fasting plasma glucose (FPG), insulin, HOMA-B, HOMA-IR, QUICKI, triglycerides, VLDL-C, LDL-C, HDL-C, total cholesterol, hs-CRP,TAC, and GSH. **Independent Variables:** daily probiotic supplementation **Control variables:** dietary intake, physical activity
Description of Actual Data Sample	**Initial:** 40 (6 male, 34 female) **Attrition:** 35 **Age:** 20-55 (average: 36.2 placebo group; 38.3 probiotic group) **Ethnicity:** Iranian **Other Relevant Demographics:** not provided **Anthropometrics:** • baseline BMI: 26.3 placebo group, 27.6 probiotic group • BMI change: 0.2 placebo group, -0.1 probiotic group **Location:** Kashan, Iran
Summary of Results	**Key Findings:** The probiotic group demonstrated significant improvements in BDI score (p=0.001), serum insulin (p=0.03), HOMA IR (p=0.03), hs-CRP (p=0.03), and GSH (p=0.02) compared to the placebo group. There was a trend towards significant improvements in HOMA-B (p = 0.06) and QUICKI (p = 0.07). There were no significant differences in FPG, lipids concentrations or TAC between the two groups. Due to significant differences in baseline FPG between the two groups, baseline age and BMI were controlled for in the analyses, which yielded no significant differences in the findings, except for BDI score (p=0.05) and serum insulin (p=0.05). **Other Findings:** There were no statistically significant differences in anthropometric characterstics of dietary intake (energy, carbohydrates, protein, fat, SFA, PUFA, MUFA, cholesterol, magnesium, manganese, zinc) between the two groups.

Author Conclusion	8 weeks of probiotic supplementation in patients with MDD resulted in beneficial effects on depression symptoms (as reflected in BDI score), insulin, HOMA-IR, hs-CRP, and GSH levels.
Reviewer Comments	• *Small sample size* • *Use of antidepressants not controlled for or discussed* • *Lack of fecal microbiome analysis*
Funding Source	Grant provided by Kashan University of Medical Sciences.

Citation	Kazemi A, Noorbala AA, Azam K, Eskandari MH, Djafarian K. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clincial trial. Clin Nutr. 2018;1-7. doi:10.1016/j.clnu.2010.04.010.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	To compare the effect of probiotic and prebiotic supplementation vs placebo on decreasing depressive symptoms, kynurenine/tryptophan (KYN:TRP) ratio, and TRP/BCAAs ratio in adult subjects with mild to moderate MDD.
Inclusion Criteria	• mild to moderate depression • age 18-50 • use of the antipressant drugs sertraline, fluoxetine, citalopram or amitriptyline for ≥3 months prior to beginning of study
Exclusion Criteria	• history of renal, hepatic, cardiovascular, or respiratory diseases • pregnancy and lactation • regular intake of probiotics during the last 2 months before study recruitment • intake of antioxidant or omega-3 supplements less than 6 weeks before beginning of study • alcohol intake • cigarette smoking (>5 during the last 6 months) or tobacco use (pipe or hookah at least one time during the last month) • opiate addication • history of heart attack or stroke • following a specific diet • participation in another study during the last two months • any significant change in diet and lifestyle • any change in drug regimen • inflammatory diseases which lasted for more than one week during the study • intake of antibiotics during the study

Description of Study Protocol	**Recruitment:** referred by psychiatrist or faculty from Tehran University of Medical Sciences in Bahman Hospital. **Design:** Randomization was stratified by age (≥35 vs <35). Participants were than randomized to groups (1:1:1) in blocks of 6. Participants were instructed to take one sachet of orally dispersible powder containing either probiotic, prebiotic, or placebo at the same time daily for a total of 8 weeks. Sachets were to be poured directly into the mouth, preferably before a meal. Participants were to follow their usual dietary intakes and physical activity, and were requested to record their dietary intake for three non-consecutive days (two usual days and one weekend day) at baseline and at study completion. Participants were monitored every 2 weeks by telephone contact. Compliance was measured by having participants return medication containers. Participants were considered to be compliant if they consumed at least 80% of their supplements. **Blinding used:** double blind **Intervention:** Daily supplementation with either 5g of an orally dispersible probiotic powder containing ten billion CFU of freeze-dried L. helveticus R0052 and B. longum R0175, or 5 g of an orally dispersible prebiotic powder containing galactooligosaccharide, for a total of 8 weeks. **Statistical Analysis:** Analysis of BDI score was completed on an ITT basis. Analysis of serum levels of KYN:TRP ratio and TRP:BCAAs ratio was completed on a PP basis, defined as those participants who completed the study. ANOVA and ANCOVA were used to compare the outcomes between groups, adjusting for corresponding baseline values for all outcomes, and BCAA and isoleucine for KYN:TRP ratio. For pairwise comparison of the groups, ANOVA and ANCOVA were also used. Kolmogorov-Smirnov test was used to test for normal distribution of the variables. Skewed data was log10 or square root transformed. When significant main effects were detected, Bonferroni multiple comparisons test was applied to compare data between groups. A sample size of at least 81 was required for 80% power with 2-sided type 1 error of 0.05 to detect a mean difference of 5 (minimal important change difference) between intervention and placebo groups. P-values <0.05 were considered significant.
Data Collection Summary	**Timing of Measurements:** BDI score (primary outcome) was measured at baseline and at study completion. Serum levels of KYN:TRP ratio and TRP:BCAAs ratio were collected as fasting blood samples (taken between 0800 and 0900) at baseline and study completion. **Dependent Variables:** Changes in BDI score and changes in serum levels of KYN:TRP ratio and TRP:BCAAs ratio. **Independent Variables:** Intake of daily probiotic or prebiotic supplement for 8 weeks. **Control variables:** dietary intake

Description of Actual Data Sample	**Initial:** 110 (32 males, 78 females)
	
	Attrition: 81
	
	Age (mean): 36.47
	
	Ethnicity: Iranian
	
	Other Relevant Demographics:
	• Education:
	  o 9% no high school certificate
	  o 26.4% completed high school
	  o 41.8% undergraduate degree
	  o 22.7% postgraduate degree
	• Duration of depression: 2.27 years (mean)
	• Duration of antipsychotic treatment: 1.72 years (mean)
	
	Anthropometrics: Mean BMI 26.5
	
	Location: Tehran, Iran

Summary of Results	**Key Findings:** ITT analysis of BDI score demonstrated significant change between groups (P=0.04). In pairwise analysis, mean BDI score significantly decreased in the probiotic group compared to the placebo group (P=0.008). Decrease in mean BDI score in the prebiotic group was not significant compared to placebo group (P=0.26). PP analysis of serum KYN:TRP ratio demonstrated no significant difference between groups. After adjusting for serum isoleucine, serum KYN:TRP ratio was significantly different between groups. In pairwise analysis of the groups, a significant decrease in KYN:TRP ratio was observed in the probiotic group compared to the placebo group (P=0.048). Change in serum TRP:BCAAs was not significantly different between groups. In pairwise analysis of the groups, a significant decrease in TRP:BCAAs ratio was seen in the probiotic group compared to the placebo (P=0.031). **Other Findings:** Baseline characteristics were similar for all three groups. Change in TRP:isoleucine was significantly different between groups (P=0.026). Adverse events were reported by 15 participants: GI complaints (4 prebiotic, 2 probiotic), nausea (1 probiotic, 1 prebiotic), fever and body aches (1 probiotic), and increased appetite (5 probiotic, 1 prebiotic). A mean compliance rate of 91.9% was achieved at study completion.
Author Conclusion	8 weeks of probiotic supplementation resulted in a greater decrease in BDI score compared to placebo. When adjusting for serum isoleucine, a signficant decrease in KYN:TRP ratio was observed between groups, specifically with a significant decrease in the probiotic group compared to placebo group. This may provide insight into the mechanisms responsible for the antidepressive properties of probiotic supplementation.

Reviewer Comments	• *Lack of response or remission rates gives little insight into whether the change in BDI score in the probiotic group was clinically significant* • *Lack of fecal microbiome analysis* • *Long recruitment phase resulting in interventions conducted during different seasons of the year (which may impact vitamin D status and lifestyle changes and ultimately dilute effect of interventions)* • *Heterogeneous treatment with antidepressant drugs* • *Diagnostic criteria for depression unclear* • *Unclear if comorbid psychiatric disorders were present*
Funding Source	Not provided

Citation	Pinto-Sanchez MI, Hall GB, Ghajar K, et al. Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: A pilot study in patients with Irritable Bowel Syndrome. Gastroenterology. 2017;153:448-459. doi:10.1053/j.gastro.2017.05.003.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	The purpose of this study was to evaluate the effect of B. longum supplementation on comorbid anxiety and depression in individuals with IBS (diarrhea or mixed stool pattern). The secondary objective was to evaluate the effect of B. longum supplementation on IBS symptoms, brain activation patterns, circulating inflammatory markers, neurotrophins, gut microbiota profile, and urine metabolites as a measure of host-microbial metabolic interactions.
Inclusion Criteria	• diagnosis of IBS with diarrhea or mixed stool pattern (Rome III criteria) • mild to moderate anxiety or depression scores based on the Hospital Anxiety and Depression (HAD) Scale (score of 8-14)
Exclusion Criteria	• history of organic diseases, immune deficiency, major abdominal surgery, or a psychiatric condition other than anxiety or depression • use of immunosuppresants, glucocorticosteroids, opioids, antidepressants, or anxiolytics in regular doses • alcohol or illicit drug consumption • antibiotic use within 3 months prior to the run-in period and during the trial
Description of Study Protocol	**Recruitment:** not provided **Design:** Participants were block randomized by gender and IBS status (diarrhea or mixed-stool pattern) to receive either 42 sachets of probiotic powder or placebo powder. Participants were instructed to take one sachet daily for six weeks. Sachets were to be mixed with 100-200 mL of lactose-free milk, soy milk, or rice milk that had been preheated to 20 degrees centigrade. The study involved 4 hospital visits. At the screening visit (-4 weeks), clinical history and symptoms were assessed, and a physical exam and complete bloodwork were peformed. At baseline visit (week 0), inclusion/exclusion criteria and symptoms were reassessed, stool, urine, and blood samples were collected, and a fMRI study was performed. At week 6, symptoms were assessed, blood, urine, and stool samples were collected, and a fMRI was performed. Symptoms were reassessed at week 10. HAD scores were also assessed at week 3 via questionnaires (originally provided at visit 1) that were returned to investigators by email or mail.

	Blinding used: double blind **Intervention:** 6 weeks of daily supplementation with a powder containing 10 billion CFU of spray-dried B. longum NCC3001 **Statistical Analysis:** Data from all subjects was analyzed on an intent-to-treat basis. To test the effect of the two primary outcomes, Pearson x^2 or Mann-Whitney U test were perfomed. ANOVA was used to analyze HAD scores at baseline and weeks 3, 6, and 10. ANCOVA was used to adjust for baseline differences in HAD depression scores. A 2-sided test was used and $P < 0.05$ was considered statistically significant. fMRI data was analyzed by a variety of methods including: general linear model, activation maps, random-effect analyses, and false discovery rate approach. Non-parametric Mann-Whitney test was used to analyze effect on biomarkers. It was estimated that 36 subjects was needed for 80% power.
Data Collection Summary	**Timing of Measurements:** Depression and anxiety were measured at 0 weeks, 6 weeks, and at follow-up (10 weeks) using the HAD-D (depression), HAD-A (anxiety), and STAI (anxiety) scales. IBS symptoms were measured at 0 weeks, 6 weeks, and 10 weeks using the Birmingham IBS score (total, constipation, diarrhea, and pain items), Quality of Life Short Form Health Survey 36 (10 items total), Patient Health Questionnaire 15 (somatization item), and Bristol Stool Scale. Inflammation markers (CRP, TNF-alpha, IFN-y, IL6, IL8, IL10, IL12 p70, IL 10/12 ratio), neurotransmitters (CGRP, substance P, serotonin), neurotrophins (BDNF), gut microbiota profile, and urine metabolites (creatine, phenylacetylglutamine, 4-cresol sulfate, trimethylamine-N-oxide), and brain activation patterns (captured via fMRI) were measured at 0 weeks and 6 weeks. **Dependent Variables:** Primary: reduction in anxiety and depressive symptoms (as measured by reduction in HAD-D and HAD-A scores by ≥2 points); Secondary: HAD scores (continuous), anxiety score (STAI), IBS symptoms, IBS global adequate relief, quality of life, brain activation patterns, inflammatory markers, neurotransmitters, neurotrophins, urine metabolites, stool microbiota profile. **Independent Variables:** daily probiotic supplementation **Control variables:** loperamide and laxative use (restricted to use as rescue medications); eating habits and fiber intake
Description of Actual Data Sample	**Initial:** 44 (20 male, 24 female) **Attrition:** 38 **Age (median):** 46.5 probiotic group (30-58 IQR), 40.0 placebo group (26-57 IQR) **Ethnicity:** • Caucasian: 86% probiotic group, 95% placebo group • Other: 14% probiotic group, 5% placebo group **Other Relevant Demographics:** • Smoking status: 14% probiotic group, 14% placebo group • Fiber consumption (g/d): 18 g probiotic group; 13.5 g placebo

	group
	• IBS subtype:
	  o Female diarrhea: 27% probiotic group, 27% placebo group
	  o Female mixed: 27% probiotic group, 27% placebo group
	  o Male diarrhea: 37% probiotic group, 32% placebo group
	  o Male mixed: 9% probiotic group, 14% placebo group
	• Baseline HAD depression score ≥8: 59% probiotics group, 36% placebo group (P < 0.05)
	
	Anthropometrics: Median BMI: 25.1 probiotics group, 24.6 placebo group
	
	Location: Ontario, Canada

Summary of Results	**Key Findings:** At 6 weeks, 14 participants in the probiotics group had HAD-D depression scores that improved by ≥2 points compared to 7 participants in the placebo group (RR 1.98, CI 1.16-3.38, P=0.04). In the per-protocol (PP) analysis, 78% of participants in the probiotics group, compared to 35% of participants in the placebo group, had lower depression scores at 6 weeks (RR 2.4, CI 1.26-4.58, P=0.016). At 10 weeks, improvement in HAD-D scores was sustained in ITT analysis (R 2.05, CI 1.07-3.93, P=0.04) and PP analysis (RR 2.14, CI 1.11-4.12), P=0.04). Results at 6 weeks were similar when analysis was performed only on patients with baseline scores indicative of depression (HAD-D ≥8). There were no signficant differences in the number of participants with decreased anxiety scores of ≥2 between groups at either 6 or 10 weeks. A sensitivity analysis revealed the beneficial effect of probiotic supplementation was more likely to occur in those participants who also reported adequate relief of IBS symptoms. **Other Findings:** There was no significant difference in continous HAD-D scores between the two groups. When baseline difference in depression score was adjusted for, there was significantly greater improvement in the probiotics group (P=0.049). No significant differences in continuous anxiety score were found between the two groups. In ITT analysis, there was no significant difference in adequate relief of IBS symptoms at 6 weeks. PP analysis revealed a significant effect in the probiotic group (RR 2.03, CI 1.13-3.65, P=0.02). This effect was not maintained at 10 week follow-up. No significant differences were found in either the overall Birmingham score or subscores at 6 and 10 weeks. No significant differences in STATI or somatization scores were observed between groups at 6 and 10 weeks. Significant improvements in QoL physical subdomain (P=0.03), general physical health (P=0.04), and problems with work or daily activities (P=0.01) were observed in the probiotics group compared to the placebo group. A greater reduction in engagement of the admygdala, frontal cortex, and temporal cortex, and a greater engagement of the occipital regions in response to fear stimuli was observed in the probiotic group. Change in engagement in the amygdala correlated with change in depression scores (P=0.004). In the probiotics group, decreased amygdala engagement correleated with decreased depression scores (P=0.03). In the probiotics group,

	reduced engagement of the amygdala was more likely to occur in the participants with adequate relief of IBS symptoms (P=0.03). There were no significant differences in serum inflammatory markers, neurotransmitters, or BDNF between groups. There were no significant differences in intestinal microbiota composition between groups. B longum was detected in 80% of participants in the probiotics group at end of treatment. The probiotics group had a signficantly lower urinary excretion of urinary metabolites (P<0.05). Levels of 4-cresol sulfate after treatment correlated with depression scores in the probiotics group (P=0.03).
Author Conclusion	6 week supplementation of the probiotic B. longum decreased depresson but not anxiety scores. Probiotic supplementation also decreased responses to fearful stimuli in multiple brain areas involved in the processing of emotions and improved overall IBS symptoms and the physical domain of quality of life. The proposed primary mechanism is modulation of host catecholamine production.
Reviewer Comments	• *Lack of a clinician-administered rating of depression and anxiety (unclear how many subjects carried formal diagnosis of MDD)* • *Unclear if participants were receiving psychotherapy during study* • *Two authors are employees of Nestec SA, two authors are employess of Nestle Institute of Health Sciences SA*
Funding Source	Funding provided by Nestle SA.

Citation	Romijn AR, Rucklidge, JJ, Kuijer, RG, Frampton, C. A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. Aust N Z J Psychiatry. 2017;51(8):810-821. doi:10.1177/0004867416686694.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	The purpose of this trial was to test whether probiotic supplementation improved depression symptoms in individuals with low mood, and to examine whether the presence or severity of IBS symptoms and levels of inflammatory biomarkers would predict or impact treatment response.
Inclusion Criteria	• Either ≥11 on the Ouick Inventory of Depressive Symptomatology (QIDS-SR16) or ≥14 on the depression subscale of the Depression, Anxiety and Stress Scale (DASS-42) • ≥16 years of age • Free of any psychiatric medication for at least 4 weeks • If receiving psychotherapy, duration of therapy must have been ≥6 months prior to trial
Exclusion Criteria	• Any neurological disorder • Renal, hepatic or respiratory disease • Any serious medical condition with major medical interventions anticipated during the trial • Pregnancy or breastfeeding • Use of any supplement considered potentially antidepressant • Serious risk of suicide or violence • Current or recent antibiotic or probiotic use
Description of Study Protocol	**Recruitment:** self-referral **Design:** Participants were randomized (1:1) to receive either a daily probiotic supplement, or a placebo that was sensorially identical, for a total of 8 weeks. Participants were asked to take one sachet of powder at the same time every day, preferably before a meal. Participants completed an online questionnaire every 2 weeks in order to monitor compliance, assess side effects, and estimate intake of various substances such as caffeine, nicotine, alcohol, and recreational drugs. **Blinding used:** double-blind **Intervention:** Daily supplementation with at least three billion CFU of freeze-dried L. helveticus R0052 and B. longum R0175 per 1.5 g sachet of orally dispersible powder for a total of 8 weeks. **Statistical Analysis:** Subjects' baseline characteristics were

	compared between groups using t-tests, chi square tests and Mann-Whitney U tests. Changes from baseline to end of treatment were compared using ANCOVA with the baseline level as the covariate. Changes in measures (i-CGI ratings) were compared using ANOVA. Analyses of all psychological outcome measures were competed on an intent-to-treat basis. Secondary analyses were completed for all psychological measures using the per protocol set (participants who took their specific product for the full intervention period). Changes in biomarker variables from baseline to end of treatment were compared using ANCOVA. Exploratory analyses were conducted using a hierarchical multiple regression to examine whether treatment response was moderated by baseline biomarker levels. All significance tests were two-tailed . P-values <0.05 were considered statistically significant. A power analysis indicated that 80 participants were needed for 80% power.
Data Collection Summary	**Timing of Measurements:** MADRS, iCGI, GAF, IBS-SSS were measured at baseline and 8 weeks; QIDS-SR16 was measured at screening, baseline, and every 2 weeks throughout the study; DASS-42 was measured at screening, baseline, and 8 weeks. IL-1B, IL-6, hsCRP, TNF-a, vitamin D, and BDNF were measured at baseline and 8 weeks. **Dependent Variables:** Changes in depression symptoms and psychological functioning (as measured by MADRS, iCGI, QIDS-SR16, GAF, IBS-SSS, and DASS-42), and serum levels of IL-1β, IL-6, hsCRP, TNF-α, vitamin D, and BDNF. **Independent Variables:** Daily probiotic supplementation. **Control variables:**
Description of Actual Data Sample	**Initial:** 79 (43 males, 36 females) **Attrition:** 69 **Age (mean):** probiotic group: 35.8, placebo group: 35.1 **Ethnicity:** • New Zealanders of European descent: 75% probiotic group, 80% placebo group • New Zealand Maori: 5% probiotic group, 3% placebo group • Other: 20% probiotic group, 18% placebo group **Other Relevant Demographics:** • History of antidepressant use: 70% probiotic group, 46% placebo group (P<0.05) • IBS severity score, median (IQR): 105 probiotic group, 75 placebo group (P<0.05) **Anthropometrics:** not provided **Location:** Canterbury, New Zealand

Summary of Results	**Key Findings:** Intent-to-treat analysis showed no significant group differences on any outcome measure. Secondary analyses showed no signficiant group differences on any primary or secondary outcome. Further analyses controlling for history of antidepressant usage, season at recruitment, presence/severity of IBS showed no significant effect on any outcome. **Other Findings:** No significant group differences were observed in change of any biomarker over the course of the study. There was no significant difference in compliance rates between groups. Exploratory analyses revealed that baseline vitamin D and change in measures of iCGI-S ($p<0.05$), QIDS-SR16 ($p<0.05$), and GAF ($p<0.05$) were signficant in the probiotic group but not the placebo group. Those individuals in the probiotic group who had high baseline vitamin D levels showed greater improvement in pscyhological outcomes than those who had low baseline vitamin D levels.
Author Conclusion	Daily probiotic supplementation for 8 weeks had no significant impact on psychological measures of depression or functioning, or on biomarkers of inflammation. Depression severity/chronicity and possible treatment resistance may have impacted these results. Baseline vitamin D levels may impact treatment efficacy.
Reviewer Comments	• *Dietary sources of probiotics were not measured or controlled for* • *Potentially relevant anthropometric variables were not measured (such as BMI, body fat percentage, and physical activity)* • *Fecal microbiome analysis was not completed to measure exposure or patient compliance*
Funding Source	Provided by Lallemand Health Solutions (manufacturer of test/placebo products); private PhD scholarship

Citation	Rudzki L, Ostrowska L, Pawlak D, et al. Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improved cognitive function in patients with major depression: A double-blind, randomized, placebo controlled study.Psychoneuroendocrinology. 2018;100:213-222. doi:10.1016/j.psyneuen.2018.10.010.
Study Design	Randomized Controlled Trial
Class	A
Quality Rating	Positive (+)
Research Purpose	To evaluate the effect of augmenting SSRI treatment with supplementation of Lactobacillus Plantarum 299v on cognitive, affective, and immune parameters of depressed patients .
Inclusion Criteria	• SSRI monotherapy or drug free at admission • Diagnosis of major depression as defined by DSM-IV-R
Exclusion Criteria	• Inflammatory, oncological, autoimmune disorders, diabetes • Previously diagnosed other psychiatric diseases other than depression • Psychoactive substance abusers • Organic brain dysfunctions • Smokers • Changes in routine blood biochemical paratmeters • Pregnancy or lactation • BMI <18.5 and >30 • Treatment with antipsychotic drugs, mood stabilizers, antibiotics, or glucocorticosteroids
Description of Study Protocol	**Recruitment:** referred from Outpatient Clinic of Stanislaw Deresz Psychiatric Hospital **Design:** Patients were randomly assigned to receive either two daily probiotic capsules or two daily placebo capsules for a total of 8 weeks. Participants were instructed to take one capusle in the morning and one capsule in the evening. Participants either continued their SSRI treatment or, if they were free from antidepressants at baseline, were started on SSRI treatment upon enrollment in the study. Participants were provided with a supply of 60 capsules for the first 4 weeks of the study. After 4 weeks, participants returned to the clinic for compliance assessment, at which time they returned their empty capsule strips. Participants were provided with the remaining supply of capsules during this visit. **Blinding used:** double blind **Intervention:** Daily supplementation with two capsules of 10 billion CFU of Lactobacillus plantarum 299v. **Statistical Analysis:** Analysis was completed on a per protocol basis. Repeated measure analysis of variance with treatment as inter-subject factors and within-subject factor time effect were performed

	for all biochemical and psychometric measurements. Statistically significant ANOVA results for treatment x time effect were further analyzed for significance with post hoc Bonferroni test. Comparison of group characterisitics were completed using Student's t-test, nonparametric Mann-Whitney U test, or chi square tests were used. The r Pearson's correlation coefficient was used to test correlations between variables. A sample size of 48 was calculated to achieve statistical power of 80% and an effect size of 0.30, with statistical significance threshold set at 0.05.
Data Collection Summary	**Timing of Measurements:** The primary outcomes, depressive and anxiety symptoms, were measured at baseline and at weeks 4 and 8, using Hamilton Depression Rating (HAM-D 17O), Symptom Checklist (SCL-90), and Perceived Stress Scale (PSS-10). Cognitive functions were measured at baseline and at study completion, using the Attent and Perceptivity Test (APT), Stroop Test parts A and B, Ruff Figural Fluency Test (RFFT), Trail Making Test (TMT) parts A and B, and California Verbal Learning Test (CVLT). To measure levels of cytokines, kynurenines, and cortisol, fasting blood samples were collected between 0800 and 0900 at baseline and study completion. **Dependent Variables:** Changes in affective symptoms (depression and anxiety), cognitive function, pro-inflammatory cytokines, kynurenines, and cortisol. **Independent Variables:** SSRI treatment with daily L. plantarum supplementation. **Control variables:**
Description of Actual Data Sample	**Initial:** 79 **Attrition:** 60 (17 male, 43 female) **Age (mean):** 38.9 placebo, 39.13 probiotic **Ethnicity:** not provided **Other Relevant Demographics:** • Education status: o Primary: 2 placebo, 0 probiotic o Vocational: 4 placebo, 9 probiotic o Secondary: 10 placebo, 7 probiotic o Higher: 14 placebo, 18 probiotic • Depression characteristics: o Recurrent: 4 placebo, 5 probiotic o Chronic (>2 years): 2 placebo, 4 probiotic • SSRI treatment before enrollment: 7 placebo, 9 probiotic **Anthropometrics:** BMI: 23.55 placebo, 24.09 probiotic **Location:** Bialystok, Poland

Summary of Results	**Key Findings:** There were no significant changes between groups in endpoints related to affective symptoms. Post hoc analysis (Bonferroni test) demonstrated significant improvement in Work Speed in the AP Test (P<0.001), CVLT levels (P=0.024), KYN concentration (P=0.017), and 3HKYN:KYN ratio (P=0.015) in the probiotic group compared to placebo. **Other Findings:** There were no significant differences in subject characteristics, psychometric parameters, or biochemical parameters between groups at baseline. There were no significant effects of interaction of treatment x time in pro-inflammatory cytokines (TNF-alpha, IL-6, IL-1-beta) and cortisol concentrations in either groups. Participants did not experience any severe side effects during the study. There were no significant differences in adverse effects between groups. All GI side effects were reported by those participants who initiated SSRI treatment upon enrollment.
Author Conclusion	Augmentation of SSRI treatment with L. Plantarum 299v improved cognitive performance and decreased KYN concentration but did not improve affective symptoms or alter pro-inflammatory cytokines or cortisol concentrations in depressed patients compared to placebo.
Reviewer Comments	• *Unclear if improvements in cognitive performance were clinically significant*
Funding Source	Provided by grants from Medical University of Bialystok, Poland

BIBLIOGRAPHY

1. Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., … Esmaillzadeh, A. (2016). Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. *Nutrition*, 32, 315-320.

2. Berg, R.D. (1999). Bacterial translocation from the gastrointestinal tract. *Advances in Experimental Medicine and Biology*, 473, 11-30.

3. Chocano-Bedoya, P.O, Mirzaei, F., O'Reilly, E.J., Lucas, M., Okereke, O., Hu, F.B., … Ascherio, A. (2014). C-reactive protein, interleukin-6, soluble tumor necrosis factor α receptor 2 and incident clinical depression. *Journal of Affective Disorders,* 163, 25-32.

4. Depression. (n.d.) In The National Institute of Mental Health. Retrieved September 29, 2017, from https://www.nimh.nih.gov/health/topics/depression/index.shtml

5. Depression. (n.d.) In World Health Organization. Retrieved October 2, 2017, from http://www.who.int/mediacentre/factsheets/fs369/en/

6. Escott-Stump, S. (2012). *Nutrition and diagnosis-related care* (7th Ed.). Baltimore, MD: Lippincott, Williams & Wilkins.

7. Ferguson, J. (2001). SSRI antidepressant medications: adverse effects and tolerability. *Primary Care Companion to the Journal of Clinical Psychiatry*, 3(1), 22-27.

8. Foster, J.A., & McVey Neufeld, K. (2013). Gut-brain axis: How the microbiome influences anxiety and depression. *Trends in Neurosciences*, 36(5), 305-312.

9. Freeman, M.P., Mischoulon, D., Tedeschini, E., Goodness, T., Cohen, L.S., Fava, M., & Papakostas, G.I. (2010). Complementary and alternative medicine for Major Depressive Disorder: A meta-analysis of patient characteristics, placebo-response rates, and treatment outcomes relative to standard antidepressants. *The Journal of Clinical Psychiatry*, 71(6), 682-688.

10. Gertsik, L., Poland, R.E., Bresee, C., & Hyman-Rapaport, M. (2012). Omega-3 fatty acid augmentation of Citalopram treatment for patients with major depressive disorder. *Journal of Clinical Psychopharmacology*, 32(1), 61-64.

11. Greenberg, P.E., Fournier, A.A., Sisitsky, T., Pike, C.T., & Kessler, R.C. (2015). The economic burden of adults with major depressive disorder in the United States (2005-2010). *The Journal of Clinical Psychiatry*, 76(2), 155-162.

12. Haroon, E., Raison, C., & Miller, A. (2012). Psychoneuroimmunology meets neuropsychopharmacology: Translational implications of the impact of inflammation on behavior. *Neuropsychopharmacology*, 37, 137-162.

13. Islam, A., Alam, F., Solayman, M., Khalil, I., Kamal, M.A., & Gan, S.H. (2016). Dietary phytochemicals: Natural swords combating inflammation and oxidation-mediated degenerative diseases. *Oxidative Medicine and Cell Longevity*, 1-25.

14. Jazayeri, S., Tehrani-Doost, M., Keshavarz, S.A., Hosseini, M., Djazayery, A., Amini, H.,…Peet, M. (2008). Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine, separately and in combination, in major depressive disorder. *The Australian and New Zealand Journal of Psychiatry*, 42, 192-198.

15. Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., … Ruan, B. (2015). Altered fecal microbiota composition in patients with major depressive disorder. *Brain, Behavior, and Immunity*, 48, 186-194.

16. Kazemi, A., Noorbala, A.A, Azam, K., Eskandari, M.H., & Djafarian, K. (2018). Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: A randomized clincial trial. *Clinical Nutrition: Official Journal of the European Society of Parenteral and Enteral Nutrition*, 1-7. doi:10.1016/j.clnu.2010.04.010.

17. Khan, A., Faucett, J., Lichtenberg, P., Kirsch, I., & Brown, W. (2012). A systematic review of comparative efficacy of treatments and controls for depression. *PLoS One*, 7 (7), e41778.

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

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

20. Lopez-Legarrea, P., de la Iglesia, R., Abete, I., Bondia-Pons, I., Navas-Carretero, S., Forga, L., … Zulet, M.A. (2013). Short-term role of the dietary total antioxidant capacity in two hypocaloric regimes on obese with metabolic syndrome symptoms: the RESMENA randomized controlled trial. *Nutrition & Metabolism*, 10(22), 1-11.

21. Lopresti, A., Maes, M., Meddens, M.J.M., Maker, G.L., Arnoldussen, E., & Drummond, P.D. (2014). Curcumin and major depression: A randomized, double-blind, placebo-controlled trial investigating the potential of peripheral

biomarkers to predict treatment response and antidepressant mechanisms of change. *European Neuropsychopharmacology*, 25, 38-50.

22. Lu, S.C. (2013). Glutathione synthesis. *Biochimica et Biophysica Acta*, 1830(5), 3143-3153.

23. Maes, M., Kubera, M., & Leunis, J. (2008). The gut-barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuroendocrinology Letters*, 29(1), 117-124.

24. Major Depression Among Adults. (n.d.) In The National Institute of Mental Health. Retrieved September 29, 2017, from https://www.nimh.nih.gov/health/statistics/prevalence/major-depression-among-adults.shtml

25. Miller, A., Maletic, V., & Raison, C. (2009). Inflammation and its discontents: The role of cytokines in the pathophysiology of major depression. *Biological Psychiatry*, 65(9), 732-741.

26. Mischoulon, D., Papakostas, G.I., Dording, C.M., Farabaugh, A.H, Sonawalla, S.B., Agoston, M., …Fava, M. (2009). A double-blind, randomized controlled trial of ethyl-eicosapentaenoate for major depressive disorder. *The Journal of Clinical Psychiatry*, 70(12), 1636-1644.

27. Mischoulon, D., Nierenberg, A.A., Schettler, P.J., Kinkead, B.L, Fehling, K., Martinson, M.A., & Hyman Rapaport, M. (2015). A double-blind, randomized controlled clinical trial comparing eicosapentaenoic acid versus docosahexaenoic acid for depression. *The Journal of Clinical Psychiatry*, 76(1), 54-61.

28. Monoaminergic. (n.d.). In Oxford Dictionaries. Retrieved April 24, 2018, from https://en.oxforddictionaries.com/definition/monoaminergic

29. Nahin, R.L., Barnes, P.M., & Stussman, B.J. (2016). Expenditures on complementary health approaches: Unites States, 2012. *National Health Statistics Report*, 95, 1-12.

30. Perez-Cornago, A., de la Iglesia, R., Lopez-Legarrea, P., Abete, I., Navas-Carretero, S., Lacunza, C., … Zulet, M.A. (2014). A decline in inflammation is associated with less depressive symptoms after dietary intervention in metabolic syndrome patients: A longitudinal study. *Nutrition Journal*, 13(36), 1-9.

31. Pinto-Sanchez, M.I., Hall, G.B., Ghajar, K., Nardelli, A., Bolino, C., Lau, J.T, …Bercik, P. (2017). Probiotic Bifidobacterium longum NCC3001 reduces depression scores and alters brain activity: A pilot study in patients with Irritable Bowel Syndrome. *Gastroenterology*, 153, 448-459.

32. Purdie, J. (2016). What is anhedonia? Retrieved from https://www.healthline.com/health/depression/anhedonia#symptoms

33. Romijn, A. R., Rucklidge, J.J., Kuijer, R.G., & Frampton, C. (2017). A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. *The Australian and New Zealand Journal of Psychiatry*, 51(8), 810-821.

34. Rudzki, L., Ostrowska, L., Pawlak, D., Malus, A., Pawlak, K., Waszkiewicz, N., & Szulc, A. (2018). Probiotic Lactobacillus Plantarum 299v decreases kynurenine concentration and improved cognitive function in patients with major depression: A double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology*, 100, 213-222.

35. Sanchez-Villegas, A., Martinez-Gonzalez, M.A., Estruch, R., Salas-Salvado, J., Corella, D., Covas, M.I., …Aros, F. (2013). Mediterranean dietary pattern and depression: the PREDIMED randomized trial. *BMC Medicine*, 11(208), 1-20.

36. Sepehrmanesh, Z., Kolahdooz, F., Abedi, F., Mazroii, N., Assarian, A., Asemi, Z., & Esmailzadeh, A. (2016). Vitamin D supplement affects the beck depression inventory, insulin resistance, and biomarkers of oxidative stress in patients with major depressive disorder: A randomized, controlled clinical trial. *The Journal of Nutrition*, 146, 243-248.

37. Simon, S. (2017). Unipolar depression in adults: Choosing initial treatment. *UpToDate*. Retrieved December 2, 2018, from https://www.uptodate.com/contents.unipolar-major-depression-in-adults-choosing-initial-treatment

38. Steenbergen, L., Sellaro, R., van Hemert, S., Bosch, J., & Colzato, L.S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, Behavior, and Immunity*, 48, 258-264.

39. Winerman, L. (2017). By the numbers: The cost of treatment. *American Psychological Association*, 48(3), 80-81.

40. Zhang, J., & An, J. (2009). Cytokines, Inflammation and Pain. *International Anesthesiology Clinics*, 45(2), 27-37.