Microbiota and Mental Health: A randomized controlled trial of dietary improvement and microbiome changes for adults with major depressive disorder depression or anxiety disorders

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Abstract

It is established that physical health and diet are interconnected, but within the recent years that has been a surge of research looking at the connections between mental health and dietary patterns. The purpose of this proposal is to determine if there is connection between dietary intervention, mental health and the gut microbiome. It is hypothesized that individuals in the dietary intervention group will significantly decrease their symptom severity scores for anxiety and depression and will significantly changes in the microbiome populations compared to the habitual diet group. The six-week trial will have 150 participants taking part in the modified Mediterranean diet intervention (DI) or following their habitual diet (HD). During this time psychological symptom severity will be scored and stool samples collected at base line, week three, and week six. Anticipated results are that the DI group will have significantly lower symptom severity and significantly different microbiome populations compared to the HD group with P=<0.001. The proposed study will provide value in determine dietary recommendations for those diagnosed with mental health disorders through subjective and objective markers. Participants will be able to participate in this study will continuing to receive therapeutic treatment.

Keywords: mental health, microbiome, dietary intervention, anxiety, depression.

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Chapter 1: Introduction to the Study

Despite a well-established connection between diet and physical health, there is a notable lack of research on the corresponding relationship between diet and mental health. Consequently, there are few dietary guidelines for the treatment of mental illness that are not related to eating disorders, which has led to a surge of research working to better understand the complexities of diet and mental health's relationship. Mental health is defined as individual's emotional, psychological, and social well-being (Nelson, 2017), whereas mental illness is defined as a range of disorders that negatively impact behaviors, emotional regulation, and cognitive abilities (Nelson, 2017). Within the realm of nutritional research, there has been an increasing number of clinical trials investigating the gut-brain-axis to determine if the microbiome may have an impact on an individual's mental health.

Background

Microbiome, in context of the collected research, refers to the bacterial and fungal populations residing within the human gastrointestinal tract. Recent research studies conducted by Karl et al. (2017) and Nishida et al. (2019) found physical and mental stress decreased diversity and lowered populations of health promoting bacteria. Jiang et al. (2020) found that the microbiomes of individuals with major depressive disorder (MDD) or bipolar disorder had increased populations of harmful bacteria, and similarly to Karl et al. (2017) and Nishida et al. (2019) found decreased bacterial diversity in the individuals' microbiomes. Research has been looking to see what factors change the microbiome such as stress, mental health illness, and dietary intake.

Dietary interventions such as changing quantities of whole grains, fruits, and vegetables, have been studied to see how they impact the microbiome. Vanegas et al. (2017), Kopf et al.

(2018), and Hiel et al. (2019) found that increasing whole grains, fruit, and vegetables improved diversity and population size of bacteria associated with good gut health. Findings from Vanegas et al. (2017) and Hiel et al. (2019) indicated it takes a timeframe of two to three weeks for dietary changes to impact the microbiome.

Dietary interventions have also been used in research to study how dietary patterns impact perceived mental health. Large general population surveys have tracked dietary intake broken down into self-reported food intake and feelings of depression for individuals that did not have a diagnosis of MDD or an anxiety disorder. These studies found overall that lower intake of fruits and vegetables were associated with higher self-reported depression and anxiety symptoms. Some studies included psychological anxiety and depression assessments to determine how dietary interventions impacted mental health.

In the Jacka et al. (2017) SMILES Trial, improvements were noted in psychological test scores for individuals with anxiety or depression when a modified Mediterranean diet intervention was implemented. A similar study based off of the SMILES studies research design was conducted by Francis et al. (2019) for individuals with MDD or anxiety diagnoses, utilized dietary education videos of the modified Mediterranean diet and noted similarly improved psychological test scores over a shorter intervention period.

Problem Statement

Despite the surge in research examining the connections between the microbiome and mental health, microbiome and dietary intervention, and dietary intervention and mental health, there appears to be a lack of research that has been conducted to examine relationships between all three.

Purpose of the Study

Using a single blind clinical trial model, this study will explore the relationship between the microbiome and depressive and/or anxiety symptom severity before, during, and upon completion of a modified Mediterranean diet dietary intervention.

Research Question:

To what extent does altering the microbiome in individuals diagnosed with major depressive disorder or anxiety through improved diet quality, impact symptom severity on anxiety and depression assessment scores?

Hypotheses:

H_o: Altering the microbiome in individuals diagnosed with MDD or anxiety, through improved diet quality, does not impact symptom severity on anxiety and depression assessment scores.

H_a: Altering the microbiome in individuals diagnosed with MDD or anxiety, through improved diet quality, reduces symptom severity on anxiety and depression assessment scores.

Sub Question 1:

Does improving diet quality in individuals diagnosed with MDD or anxiety, by following a modified Mediterranean diet, alter microbiome bacterial populations?

Hypotheses

H_{o1}: For individuals diagnosed with MDD or anxiety, improving diet quality by following a modified Mediterranean diet does not alter microbiome bacterial populations.

H_{a1}: For individuals diagnosed with MDD or anxiety, improving diet quality by following a modified Mediterranean diet significantly increases beneficial microbiome bacterial populations.

Sub Question 2:

Does improving diet quality in individuals diagnosed with MDD or anxiety, through a modified Mediterranean diet, impact symptom severity on anxiety and depression assessment scores?

Hypotheses

H_{o2}: Improved diet quality achieved by following a modified Mediterranean diet has no effect on the severity of depression and/or anxiety symptoms in individuals diagnosed with MDD or anxiety.

H_{a2}: Improved diet quality achieved by following a modified Mediterranean diet reduces the severity of depression and or anxiety symptoms in individuals diagnosed with MDD or anxiety.

Theoretical Framework:

The proposed trial will operate under the Vector Model of Complexity proposed by Safford, Allison, & Kiefe (2007). The Vector Model of Complexity highlights the various factors that impact an individual's response to recommended medical treatment by acknowledging variables of culture, socioeconomics, biology/genes, environment, and behavior. With the proposed study focusing on dietary intervention impact on mental health and the microbiome, it will be important to consider how the complexity of individual participants will impact compliance with dietary recommendations as well as ability to make progress within therapeutic treatment.

Microbiome changes will also need to be reviewed under the lens of the Vector Model of Complexity as Lam, Zhang, & Zhao (2018) discussed how the microbiome changes in response to dietary changes as well as weight shifts. The proposed microbiome nutrition concept

highlights the need for further study of how the microbiome impacts an individual's health status with certain bacterial guilds (or bacteria that co-occur in the same ecosystem) impacting the metabolic pathways within an individual's body. Data collected on the microbiome will need to be analyzed acknowledging the factors of dietary compliance, medications, and socioeconomic status.

Nature of Study

A detailed examination of the proposed clinical study can be found in Chapter 3. To summarize adult participants enrolled in Madison's Intensive Outpatient (IOP) program will be randomly assigned to either the dietary intervention or the habitual diet group. Those in the dietary intervention group will be educated on the modified Mediterranean diet. Nutrition education will be provided by both pre-recorded video and dietary counseling from registered dietitian nutritionists. Participants will collect stool samples for 72-hour periods at baseline, three weeks, and six weeks. Analysis will be completed by the University of Wisconsin-Madison Biotechnology Center.

Participants will take the Diet History Questionnaire III at baseline and at the start of week 6 to assess diet quality and to assign a diet quality score. Three 24-hour diet recalls will be collected at the end of week three. Statistical testing will include t-tests to analyze change between individual participants for how diet quality impacts depression and anxiety scores, and how diet quality impacts the microbiome bacterial populations. A MANOVA test will be used to analyze the impact of change in diet quality on the psychological test scores and microbiome bacterial populations.

Definitions

Microbiome: The bacteria and fungi microorganisms that reside in the gastrointestinal tract of humans.

Alpha-diversity: The diversity of species within a particular area or ecosystem that is expressed through the number of species present (Oxford University Press, 2021). Within context of this research study, it will pertain to the number of microorganism species within an individual's gastrointestinal microbiome.

Diet Quality Score: The score assigned to rate how closely the individuals' dietary habits compare to the dietary recommendations of the Modified Mediterranean Diet.

Modified Mediterranean Diet: A dietary pattern that recommends five to eight servings of whole grains, three servings of fruit, six servings of vegetables, three to four servings of legumes, two to three servings of low fat unsweetened dairy products, one serving of raw and unsalted nuts, and three tablespoons of olive oil per day. Further dietary recommendations include two servings of fish, three to four servings of lean red meat, two to three servings of chicken, and up to six servings of egg per week.

Intensive Outpatient (IOP): Therapy which is provided by meeting with a therapists five days a week for three-hours of individual, group, and family therapy sessions.

Major Depressive Disorder (MDD): Meeting the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) which includes exhibiting 5 of the following symptoms: depressed mood by participant report or observation of others, anhedonia (inability to feel pleasure), change of more than 5% of usual body weight within 1 month without purposeful weight loss or weight gain, insomnia or hypersomnia daily, daily fatigue, feelings of worthlessness or excessive guilt, psychomotor agitation or retardation as observed by others,

decreased ability to concentrate, indecisiveness, or recurrent suicidal ideation or suicide attempts. These symptoms impact the individual's ability to function in social or occupational settings, and they cause significant distress. (DSM-5, pg. 160-161) See Appendix C for full diagnostic criteria of MDD.

Generalized Anxiety Disorder (GAD): Meeting the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) which includes exhibiting three of the following symptoms: excessive anxiety or worry over many activities occurring more days that not over a six-month period, restlessness or feeling on edge, easily becoming fatigued, difficulty concentrating, irritability, muscle tension, and sleep disturbance. These symptoms impact the individual's ability to function in social or occupational settings and cause significant distress. (DSM-5, pg. 222). See Appendix D for full diagnostic criteria of GAD and other anxiety disorders.

Assumptions

- The gut microbiome has an impact on depression and anxiety.
- Participants will answer depressive and anxiety symptom severity and diet quality questions accurately.
- Participants will take medication as prescribed and follow existing therapeutic recommendations.
- Financial incentive provided in the form of a Visa®gift card will be used to cover additional grocery expenses.
- Participants will have access to a grocery store, as well as locations to safely store and prepare food.

Limitations

- The study will be limited by small sample size due to recruiting from one clinic
- Participant's medication regimen and other therapeutic treatments may impact appetite, mood, and motivation.

Delimitations

- This study will exclude pregnant individuals, those who have history of eating disorders or metabolic diseases, and individuals with medical conditions impacted by diet.
- This study is limited to individuals with a diagnosis of MDD or anxiety disorders. Individuals with other mental health diagnoses will be excluded.
- This study is limited to individuals age eighteen or older, enrolled in an IOP program in the Rogers Behavioral Health Madison Clinic, and receiving psychological treatment for the duration of the study.

Significance

Determining potential connections between microbiome, mental health, and dietary intervention could contribute to making dietary recommendations for those diagnosed with MDD or anxiety disorders. Including the microbiome in a study design similar to Jacka et al (2017) SMILES trial could provide objective data to showcase how dietary intervention can impact mental health. This could lead to the creation of dietary recommendations and interventions targeting those with mental health issues similar to how dietary recommendations exist for other diseases such as hypertension or cardiovascular disease.

Summary

The proposed research study will look at the potential relationship between dietary interventions, mental health, and the microbiome. With limited research conducted on the impact

of dietary interventions on individuals with mental health diagnoses, further research within this area will help create dietary recommendations for these individuals. The use of the microbiome data will provide objective data via changes in microbiome bacterial populations that are associated with dietary interventions and further the research already conducted examining the impact between the microbiome and mental health. The next chapter will review the existing research encompassing mental health, dietary intervention, and the microbiome.

Chapter 2: Review of Literature

"An apple a day keeps the doctor away" is a well-known phrase dating back to 1866 (Davis et al, 2015). While increased apple consumption was not associated with limited doctor visits within the twenty-first century, as found in Davis et al. (2015) study, the association between food and overall health is well acknowledged throughout the world. Health organizations have worked to educate the public on the importance between diet and overall health using tools like MyPlate or country-specific dietary guidelines (U.S. Department of Agriculture). Despite a well-established connection between diet and physical health, there is a lack of research on the corresponding relationship between diet and mental health. Consequently, there are few dietary guidelines for the treatment of mental illness, which has led to a surge of research working to understand the complexities between the diet-mental health relationship (DMHR) and gut-brain-axis (GBA) within the field of Nutritional Psychology (Nutrition Psychology, 2020). The GBA is the interaction between the gastrointestinal tract and the nervous system; research indicates the gut microbiome influences intestinal digestion, immune responses, intestinal structure, and it may influence behaviors and stress responses (Nutrition Psychology, 2020). To better understand the DMHR and its relationship to the GBA, this literature review examines the connection between dietary interventions for individuals with mental health diagnoses and the corresponding impact on the microbiome. This is done by first examining the difference in the microbiomes of individuals with mental health diagnoses and healthy individuals acting as the control. Once baseline data has been assessed, researchers then examined how dietary interventions impacted the microbiome in those with mental health diagnoses compared to healthy individuals. The literature review also explores the connection

between dietary patterns and reported mental health conditions and examines the results of dietary interventions targeting changes in mental health.

For this literature review, studies were collected utilizing similar search terms in various online research portals including Google Scholar, PubMed, Science Direct, and Mount Mary University's library system. Search parameters for each article included that the article was written within the past 5 year as of 2020, was a clinical trial or clinical study, and utilized human subjects. Search phrases utilized included the following: "Gut Microbiota AND Mental Health," "Mental Health AND Food," "Nutrition AND Mental Health," "Mental Health AND Diet," "Gut Microbiota AND Nutrition," "Nutrition AND Mood," "Mental Health AND gut brain connection," and "Mental Health AND Microbiome." Use of these search terms revealed several repeating studies. Resources related to the Ancel Keys Minnesota Starvation Study were obtained from the Clinical Nutrition Department of Rogers Behavioral Health of Oconomowoc Wisconsin.

Mental Health and Physical Wellbeing

Mental health is defined as an individual's emotional, psychological, and social well-being (Nelson, 2017). Mental illness is a range of disorders that negatively impact behaviors, emotional regulation, and cognitive abilities (Nelson, 2017). Examples include depression, anxiety, obsessive compulsion disorder, eating disorders and addictive behaviors (Nelson, 2017). Individuals suffering from mental health concerns fall onto a spectrum of severity depending on how much the mental health disorder impacts their ability to function in their daily lives. According to the National Institute of Mental Health (NIMH), approximately one in every five Americans has a mental health condition (NIMH, 2019), and since the global COVID-19 pandemic, Americans are increasingly reporting anxiety and depression symptoms (Mental

Health America, 2020). NIMH (2019) reported that only 42.6% of those with diagnosed mental health conditions have sought treatment in 2017, leaving many without resources to manage their health and lives. Mental health continues to be a longstanding issue; however, there is still a gap in our knowledge in regard to the development of mental illness. This has led many researchers to look at the connection between psychological health and physical health stemming from the microbiome, the population of bacteria within the human gastrointestinal tract.

Minnesota Starvation Study

Mental health was first documented to be connected to physical wellbeing seventy-five years ago. As World War II raged across the globe, a historical study conducted in Minnesota provided groundbreaking data for the fields of nutrition and psychological health. With the intent to provide guidance for the rehabilitation of people in starving war-torn countries, primary researcher Ancel Keys had thirty-six young men take part in an eleven-month-long study to determine the physiological and psychological impact starvation has on humans (Kalm & Semba, 2005). Participants had a six-month semi-starvation period during which they consumed approximately 1800 calories per day and met various physical demands, such as walking twenty-two miles per week as cited in Kalm & Semba, 2005. As the starvation phase progressed, the participants' weights decreased, and they developed new behaviors indicating psychological changes.

The men exhibited emotional, sexual, social, and cognitive changes as well as changes to their personality and eating behaviors as cited in Kalm & Semba, 2005. The men reported decreased cognitive abilities, evidenced by self-perceived decreases in concentration, comprehension, mental acuity and how they perceived their work to be affected by starvation. Prior to the study, the participants had no history of mental illness; however, many of the men

developed depression, anxiety, and increased irritability throughout the duration of the study (Kalm & Semba, 2005). Intelligence testing found no indication of decreased intelligence, though, as cited in "Starvation and Behavior" (2017). Despite cognitive tests not providing any significant changes in cognitive abilities, the fact that participants indicated decreased cognitive abilities could have likely been tied to their overall mental state.

Changes to the men's eating behaviors included increased food preoccupation, creating unappetizing food concoctions, sneaking food, ritualistic eating behaviors, increased consumption of items like coffee, tea, and gum ("Starvation and Behavior," 2017). Several men also started to develop habits indicating what is now known as binge eating; the men would eat foods in such large quantities that they would vomit as cited in "Starvation and Behavior" (2017).

While Keys' study provided a foundation for the treatment of eating disorders, it also showed a significant connection between starvation and mental health through documented changes in behaviors for the participants ("Starvation and Behavior", 2017). There has been significant advancement in the treatment of eating disorders since Keys' Starvation Study, but there is a severe lack of information on the role of nutritional intervention in general mental health and non-eating disorder mental health diagnoses.

Mental Health's Impact on the Microbiome

The results of the Starvation Study showed that rapid changes in the participants' diets to reflect that of war-torn Europe impacted the men's eating behaviors and led to the development of depression and anxiety (Kalm & Semba, 2005). The subsequent weight loss showed the impacts of starvation on mental health; the same symptoms are observed in patients who engage in eating disorder behaviors. Individuals diagnosed with eating disorders can have very rigid

rules surrounding what types of foods they are able to eat (Gaudiani, 2019), which can significantly limit what foods individuals are willing to consume. Significant dietary changes, which can be observed during periods of illness or environmental causes, impact overall nutritional health and can impact the state of the microbiome. Scientists have started to research effects of dietary change on the human body through the microbiome, which is the microorganisms that reside in the gastrointestinal tract of humans. Recent research of the microbiome has looked at both its impact on overall health as well as how it is affected by physical and mental illness. The microbiome is formed through different types of bacteria, archaea, eukaryotes, and fungal microorganisms that change throughout an individual's lifetime in coordination with lifestyle patterns, medication, dietary intake, and illness (Kim & Shin, 2018). Table 1 highlights significant bacteria and briefly indicates the impact on the microbiome. The microbiome breaks down fiber, releases metabolites, and sends chemical signals locally to the GI tract and the overall nervous system.

Duvallet et al. (2017) compared microbiomes in individuals with ten different diseases in a meta-analysis to understand the impact of disease on the microbiome. The researchers aggregated the data from twenty-eight published studies and compared the data across specific disease states to determine what changes occurred in the bacterial communities in relation to healthy control participants.

Their cross analysis of four studies pertaining to irritable bowel disease (IBD), specifically Crohn's and Ulcerative Colitis, shows that compared to healthy control populations, those with IBD have decreased populations of *Clostridiales, Ruminococcacaea*, and *Lachnospiraceae* bacteria, which are associated with overall gut health (Duvallet et al., 2017). The *Ruminococcacaea* and *Lachnospiraceae* bacteria families are short-chain fatty acid (SCFA)

producers, while *Clostridiales* specifically produce butyrate (Duvallet et al., 2017). SCFA's, such as acetic, propionic, and butyric acid, are all important in maintaining colon health due to their role in GI hormone productions, reproduction of colonocytes, and stimulation of autonomic nervous system functions in the colon (Kim & Shin, 2018). IBD has a high correlation with anxiety and depression, with over 50% of affected individuals also being diagnosed with either anxiety or depression (Kim & Shin, 2018).

While Duvallet et al. (2017) reviewed how physical illnesses are associated with the bacterial populations of the microbiome, Borgo et al. (2017) compared the differences between healthy individuals and those diagnosed with anorexia nervosa (AN). AN is defined by extreme energy restrictions leading to significant weight loss with the potential for being at a dangerously low body weight; individuals diagnosed with AN also regularly have anxiety or depression (Borgo et al., 2017). Using data from fifteen participants diagnosed with AN and fifteen control participants, Borgo et al. (2017) examined differences in participants' microbiomes. Dietary data, stool, and blood samples were all collected. Additionally, all participants took the following psychological tests: Symptom Checklist-90, Eating Disorder Inventory 2 (EDI-2), State Trait Anxiety Inventory Scale (STAI), and Beck Depression Inventory (BDI-II). The AN group had significantly decreased levels of *Firmicutes,* specifically *Ruminococcus, Roseburia* and *Clostridium,* compared to the control group (Borgo et al., 2017). The decreased amount of *Roseburia* bacteria is associated with the significantly lower level of SCFA butyrate because *Roseburia* produces butyrate as a metabolite. Compared to the control group, the AN group had significantly increased levels of *Proteobacteria* and *Enterobacteriaceae*; *Enterobacteriaceae* have been associated with increased inflammation throughout the body (Borgo et al., 2017). Borgo et al. (2017) had significant findings in the differences between the microbiomes of

healthy individuals and those diagnosed with AN, but the study overall had a very limited sample size. Individuals actively engaging in eating disorder behaviors or starting the recovery process often have select 'safe' foods that they are willing to eat before reincorporating other foods into their diet (Gaudiani, 2019). The limited variety of foods that those with AN typically consume is a probable explanation for the decreased levels of beneficial SCFA-producing bacteria (Borgo et al. 2017).

As seen in Borgo et al. (2017) and Duvallet et al. (2017), physiological and psychological conditions impact the microbiome's bacterial populations. Compared to the effects of existing medical conditions, Karl et al. (2017) chose to see how temporary stress impacted the microbiomes of healthy young adults. Karl et al. (2017) examined changes to the microbiomes of young adults experiencing physiological stress and found that increased stress alters the populations of microbiota associated with inflammation. Data was collected on seventy-three military personnel prior to taking part in fifty-one-kilometer cross-country ski-marches while carrying a forty-five-kilogram pack of equipment. The study included three diet groups: control group, which was only provided military rations; protein group, which was provided an additional four whey-protein snack bars; and carbohydrate group, which was provided an additional four carbohydrate-based snack bars (Karl et al., 2017). The researchers found that multiple forms of stress, presented as physical exertion, inadequate energy intake, and inflammation, negatively impacted the microbiome. Stool samples taken either the night of or the morning after stress found increased amounts of less dominant bacteria when compared to stool samples taken prior to the march. The bacteria *Peptostreptococcus, Staphylococcus, Peptoniphilus, Acidaminococcus,* and *Fusobacterium* were associated with decreased immune protections regardless of dietary intervention. Duvallet et al. (2017) identified that

Peptostreptococcus is considered a pathogenic or pathogen-associated bacterium and is associated with microbiome dysbiosis.

Decreased intestinal permeability in times of physiological stress may be linked to microbiome changes. Karl et al. (2017) reviewed that *Bifidobacterium* and *Collinsella* are associated with anti-inflammatory effects and are commonly found in probiotics, which may have a protective factor for those under stress. *Bifidobacterium* is associated with intestinal health because of its interactions with the immune response, which aids in the prevention of infections. It also produces beneficial health metabolites, which can promote anti-inflammatory effects, allowing it to have beneficial effects for those with physiological stress symptoms (Nishida et al, 2019). Karl et al. (2017) discussed that while their study examined the impact of physical stress on the microbiome, it does not consider the psychological or sleep-deprivation stress that participants likely went through and is focused on with Nishida et al. (2019).

Nishida et al. (2019) examined the impact of *Lactobacillus gasseri* CP2305 tablets on sixty psychologically stressed Japanese young adults in medical school. The double-blind test took place over twenty-four weeks, during which participants took two tablets of *Lactobacillus* or placebo daily; the *lactobacillus gasseri* CP2305 tablets contained 1.0×10^{10} bacterial cells for the two-tablet dose. The changes of the microbiome were monitored by collecting stool samples two weeks prior to intervention starting and at the end of the twenty-four-week intervention period. Mental health was assessed using the Spielberger State-Trait Anxiety Inventory (STAI), the 28-item General Health Questionnaire (GHQ-28), and the Hospital Anxiety and Depression Scale (HADS) two weeks prior to interventions starting, at week twelve, and at the end of the twenty-four-week intervention period. The results indicated that those in the *Lactobacillus gasseri* group experienced a significant decrease in STAI-trait anxiety scores and GHQ-28

depression scores compared to the control group. HADS indicated that the *Lactobacillus* group had improved depression and anxiety scores compared to the control group, but this was not considered clinically significant (Nishida et al. 2019). Researchers also found that the *Lactobacillus gasseri* group had a significant increase of Valeric-acid, as SCFA, compared to the control group. The *Lactobacillus gasseri* tablet supplementation significantly decreased stress-related reduction of *Bifidobacterium* colonies in the microbiome and mitigated the increase of *Streptococcus* bacteria, which is associated with colorectal cancer (Nishida et al. 2019). Nishida et al. (2019) identified that psychological stress had an impact on the bacterial population within young adults who had no active mental, inflammatory, bone, or hormonal diseases.

Karl et al. (2017) also found that physiological stress impacted the microbiomes of otherwise healthy military recruits. Stress, physiological, and psychological conditions are shown to negatively impact the microbiome, with stress both reducing health-promoting bacteria *Bifidobacterium* (Nishida et al, 2019) and increasing populations of pathogenic- or illness-associated bacteria (Karl et al. 2017; Nishida et al. 2019). Furthermore, AN and IBD have a negative impact on the bacterial population of SCFA producers (Borgo et al., 2017; Duvallet et al., 2017). To better understand the impact mental health has on the microbiome, Jiang et al. (2020) and Severance et al. (2017) examined the differences in the microbiome for those with depression and schizophrenia.

Jiang et al. (2020) compared the microbiomes of sixteen healthy individuals without any history of mental illness to twenty-four individuals experiencing a depressive episode or diagnosis of bipolar disorder. None of the participants took medication that interacts with the bacterial-fungal populations of the gastrointestinal tract during the two months prior to sample collection. There was no significant difference in the microbiome between bipolar disorder or

depression, nor with the use of antidepressants. When comparing the microbiomes of those with depression or bipolar disorder to the healthy controls significant differences existed within the microbiota populations. The fungal microbiota in those diagnosed with bipolar disorder or depression had increased fungal populations of *Candida,* and decreased fungal populations of *Penicillium,* compared to the healthy participants. Jiang et al. (2020) found that the mental health disorder patients had decreased populations of beneficial bacteria and increased harmful bacterial populations. Jiang et al. (2020) indicated that the healthy individuals have complex connections between the diverse microbiota compared to those with the mental health conditions, which reinforces the work of Nishida et al. (2019) that showed greater diversity in the microbiome of healthy individuals.

Jiang et al's. (2020) results indicate that those with bipolar disorder or depression have an increased presence of *Candida albicans*, the bacteria commonly associated with yeast infections. Increased levels of *Candida albicans* is associated with increased GI discomfort in individuals with schizophrenia (Severance et al., 2017). Severance et al. (2017) provided fifty-six male patients with schizophrenia "Bifiform Balance," a probiotic containing *Lactobacillus rhamnosus* and *Bifidobacterium animalis*, or a placebo pill for fourteen weeks. Participants ranked bowel health on a 1-4 spectrum of self-assessment questions relating to ease-of-bowel movements. The probiotic was found to decrease the presence of *Candida albicans* and improve overall bowel health compared to the control group (Severance et al., 2017).

While several of the reviewed studies found significant connections between psychological disorders and physical health (Borgo et al., 2017; Jiang et al., 2020; Karl et al., 2017; Nishida et al., 2019, and Severance et al., 2017) all were done with small sample sizes, which limited the validity of the results. Although small sample size can increase risk for data

being skewed, the findings from Jiang et al. (2020), Borgo et al. (2017), Severance et al. (2017), Karl et al. (2017), and Nishida et al (2019) reinforce results discussed throughout each of their respective studies.

Studies by Borgo et al. (2017), Jiang et al. (2020), and Severance et al. (2017) provided evidence that microbiomes of individuals with mental health disorders have different bacterial populations, while Karl et al. (2017) and Nishida et al (2019) provided evidence that stress negatively impacts the bacterial populations of the microbiome. While the studies indicate that stress and mental health conditions contribute to changes in the bacterial and fungal populations in the microbiome, are these differences related to the impact of stress and or the mental health conditions, or do other factors, such as dietary intake, play a role? Severance et al. (2017) and Nishida et al. (2019) found that probiotic interventions acted as protective factors in maintaining healthy gut bacteria. Prebiotics, which encourage growth in bacteria, may be beneficial in providing protection to the microbiome during periods of stress or illness. Foods that are classified as prebiotics have a high-fiber content and serve as the food for the microorganisms within the GI tract. Common examples of prebiotic foods are whole grains, fruits, vegetables, and legumes, while probiotics, which are items containing microorganisms, are fermented items like yogurt, sauerkraut, or kombucha (Klemm, 2020).

Dietary Interventions impact on the Microbiome

Fermented Dairy

Several studies have been conducted to analyze how dietary interventions of increasing fermented milk, whole grains or vegetables impact the microbiome as compared to the control diet. Uemura et al. (2019) provided twenty-two Japanese women dietary interventions focusing on promoting microbiome health by increasing intake of vegetables and fermented dairy

products. Stool samples were collected from the women before and after the eight-week intervention period for comparison to the twenty-two control participants. The dietary intervention group received four nutrition education sessions led by registered dietitians followed by a counseling period and were found to significantly increase their consumption of vegetable dishes and milk products compared to the control group (Uemura et al., 2019). In addition, they had significantly decreased *Bacteroidaceae,* and significantly increased *Lactobacillales*, *Bifidobacterium bifidum, Streptococcus thermophiles,* and *Veillonella parvula* populations compared to the control group (Uemura et al., 2019). *Bifidobacterium bifidum* and *Veillonella parvula* are both SCFA producers that have been associated with overall colon health, increased insulin sensitivity, and energy expenditure, which the researchers identify as a potential reason for the significant weight, waist circumference, and BMI reduction in the dietary intervention group (Uemura et al., 2019). It is also important to acknowledge that these changes may have been due to the dietary changes in the participants' diets.

Bellikci-koyu et al. (2019) also focused on increasing intake of fermented dairy products, but unlike Uemura et al. (2019), interventions were designed to maintain the habitual diet with the exception of diary intake. A dietary intervention was conducted with twenty-two participants in either a kefir or unfermented milk group where participants received 180 mL of kefir or milk daily for twelve weeks. Stool samples were collected at baseline and once intervention ceased. The researchers found that Tumor necrosis factor alpha (TNF-α) and Interferon-γ (INF-γ) were significantly decreased in the kefir group, while the milk intervention had a significant decrease in Interleukin 6 (IL-6). Data shows various changes to the gut microbiota's composition; however, the changes were not considered significant between groups except for increased prevalence of the phylum *Actinobacteria* (Bellikci-koyu et al., 2019). Kim and Shin (2018)

discussed in their review of the microbiome that the decreased inflammatory markers could be related to the microbiomes influence on immune system responses. While both dietary interventions had significant decreases in inflammatory markers, there were no significant changes to the microbiome between the intervention groups.

Although Bellikci-koyu et al. (2019) found no significant differences between the microbiome bacteria populations in the kefir and milk interventions, researchers did find decreased inflammatory markers which can be associated with overall health. Additionally, Uemura et al. (2019) found that increasing vegetable and dairy products increased bacteria associated with a healthy colon. Despite small sample size, results from both Bellikci-koyu et al. (2019) and Uemura et al. (2019) indicate that increasing probiotic and prebiotic foods, such as dairy and vegetables, is associated with overall health. These results are also similarly noted in other studies that focused on increasing the consumption of prebiotic foods compared to the habitual diet of the participants prior to the intervention.

Whole Grains, Fruits, and Vegetables

Vanegas et al. (2017) examined the impacts of refined grains compared to whole grains on the microbiomes of eighty-one individuals. Participants started the trial consuming a Western-style diet for two weeks to minimize the effects of existing dietary patterns prior to the start of the study. Nutrition intervention occurred by assigning groups to be refined grains with the average daily fiber intake consumed by Americans, and whole grains meeting the Dietary Guideline for Americans recommendations. Stool samples were collected at the end of the two-week Western-diet phase and then again after the grain dietary intervention. Microbiome changes indicated that there was a significant increase in *Lachnospira* bacteria, which are SCFA producers and are associated with good colon health (Vanegas et al., 2017). Increased dietary

fiber from whole grains was also associated with a significant decrease of proinflammatory *Enterobacteriaceae*. Vanegas et al. 2017 found that there was modest positive correlation between a whole-grain diet and decreased Tumor necrosis factor alpha (TNF-α).

Vanegas et al. (2017) acknowledged that their study had lower changes in inflammation and microbiota than previous literature, but participants sought out for this study were healthy individuals who were not immunocompromised, likely leading to lower existing inflammatory responses. Vanegas et al. (2017) findings are supported by Bellikci-koyu et al. (2019) and Kopf et al. (2018) indicating that increased fermented food and whole grains are associated with decreased inflammation.

Kopf et al. (2018) conducted a similar study that had forty-nine participants randomly assigned to one of three groups: one whose fruit and vegetable intake was increased, one whose whole-grain intake was increased, and one serving as a control. The fruit and vegetable intervention increased intake to three one-cup servings daily, while the whole-grain intervention had three one-ounce servings daily. Diets were followed for six weeks. The fruit and vegetable intervention significantly increased the alpha diversity of the participants' microbiome, while the whole grain intervention did not (Kopf et al., 2018). Researchers found that increased fruits and vegetables decreased Lipopolysaccharide binding protein (LBP) and IL-6 inflammation markers; the whole-grains group decreased LBP and TNF-α. Elevated LBP indicates that the microbiome is in a state of endotoxemia, meaning that the microbiome is unable to protect itself from absorbing toxins (Kopf et al., 2018).

Hiel et al. (2019) examined the impacts of increasing inulin, a specific dietary fiber, in twenty-six healthy participants. The participants were provided one hot meal throughout the study for lunch, and then were instructed to make a high inulin soup nightly for dinner for the

two-week interventions. Stool samples were collected before and after dietary intervention. Microbiome analysis suggested that alpha diversity decreased after the intervention, meaning that there was decreased variety of bacteria found in the microbiome. However, univariate analysis found that inulin rich diets increased *Bifidobacterium* genus three-fold and significantly decreased *Clostridiales* (Heil et al., 2019). Kopf et al. (2018) found that dietary intervention increasing fruits and vegetables increased the alpha-diversity of participants' microbiome and indicated that it may be related to increased variety of dietary fibers. Since Hiel et al. (2019) intervention solely focused on inulin vegetable sources, it is possible that one type of fiber source limited the impact on the microbiome. Once the dietary intervention ended, researchers found that microbiome levels returned to pre-inulin intervention levels three weeks after normal diet resumed. This means that the dietary intervention had temporary effects on the microbiome; permanent changes to the dietary pattern would likely need to be made to maintain the positive microbiome changes. The short-term impact of the dietary intervention on the microbiome is reinforced within the methodology of the Vanegas et al. (2017) study as they had participants following a Western dietary pattern for two weeks prior to intervention in order to have similar baseline microbiome data.

Despite Vanegas et al. (2017), Kopf et al. (2018), Hiel et al. (2019), Bellikci-koyu et al. (2019) and Uemura et al. (2019) studies all being conducted with small sample sizes, they found significant changes with bacterial populations in the microbiome with several studies noting the improvement of inflammation markers. Improving dietary consumption of prebiotic foods such as vegetables was associated with increased beneficial microbiome bacteria; however, findings from Hiel et al (2019) seem to suggest that a variety of dietary fiber sources have a greater overall benefit to the microbiome. Increasing whole grains was associated with decreased

inflammation markers in Vanegas et al. (2017) and Kopf et al. (2018). Increasing dairy products was found to also decrease inflammation markers in studies by Bellikci-koyu et al. (2019) and Uemura et al. (2019), but there was no significant difference between the benefits of fermented and unfermented dairy products.

An important aspect of these studies was the indication of the timeline needed for interventions to impact the microbiome. Vanegas et al. (2017) and Hiel et al. (2019) indicated that dietary changes take approximately two or three weeks to impact the microbiome, and once the intervention stops, the bacterial population change is not permanent. Acknowledging the short-term impact of dietary interventions can impact the results of a study depending on the length of the intervention period.

Dietary Quality and Mental Health

As dietary interventions have been shown to impact the microbiome temporarily, it is important to understand overall diet quality and how it impacts an individual's mental health long term. Schweren et al. (2020) analyzed dietary patterns of 121,008 individuals in the Netherlands over 3.6 years. The data collected from a semi-quantitative food frequency questionnaire was evaluated to determine an individual's Lifeline Diet Score based on the frequency of consuming specific food groups. Participants underwent a mental health assessment by partaking in a Mini International Neuropsychiatric Interview reviewing symptoms appearing within the past six months. If criteria were met, based on the DSM-IV, participants were diagnosed with the depression or anxiety disorder. Schweren et al. (2020) defined a high diet quality as regular consumption of fruits, vegetables, whole grains, low-fat dairy products, nuts and seeds, fish, and oil, with limited consumption of red meats, similar to what is recommended when following a Mediterranean diet pattern. Overall, it was noted that diet quality increased

with age, being at a lower BMI, being female, and having higher levels of education (Schweren et al., 2020). Similarly, another study found that social conditions impacted overall heath; those who were unmarried, relatively young, or unemployed had higher incidence of depression (Bishwajit et al., 2017).

Schweren et al. (2020) found that exposure to stressful life events, such as serious illness or trauma, was significantly associated with decreased diet quality. While researchers found that poor diet quality was significantly correlated with anger-hostility, self-consciousness, impulsivity, and vulnerability, there was not a significant correlation between overall diet quality and the risk of developing anxiety or depression throughout study timeframe either in the general population or for those with existing mental health conditions. Schweren et al. (2020) reported that diet interventions conducted throughout previous clinical trials, identified in the systematic review conducted by Opie et al. (2015) likely were not associated with the development of anxiety or depression.

The large population size used in Schweren et al. (2020) allowed for data to be gathered on young, old, male, and female populations with very few individuals being excluded based on medical history; several other studies, such as Vanegas et al. (2017) and Conner et al. (2017) excluded those with other medical diseases or medications. Data of Schweren et al. (2020) provided a better representation of the general public due to the inclusivity of the study, which led to the author's conclusion that long-term dietary interventions were unlikely to contribute to decreased prevalence of anxiety or depression. Schweren et al. (2020) noted that the clinical impact of dietary interventions may not have been observed over the 3.5-year period, and as discussed earlier, Heil et al. (2019) found that the microbiome returned to pre-intervention bacterial states after the intervention stopped. Schweren et al. (2020) noted that improved mental

health with dietary changes, as seen in other clinical trials, was likely due to participant perception that increased diet quality is an act of self-care.

While Schweren et al. (2020) found that diet quality may not be indicative of the development of depression or anxiety, the association between fruit and vegetable intake and perceived mental status has been studied throughout the world. Fruits and vegetables are high in fiber as well as many vitamins and minerals which have been linked to overall physical health. Richard et al. (2015), Bishwajit et al. (2017), and Gibson-Smith et al. (2020) have looked at the connection between fruit and vegetable intake by adults in several different regions in population-based studies from across the globe.

Richard et al. (2015) utilized data from the Swiss Health Survey to examine trends between mental health and fruit and vegetable consumption. Mental health was assessed in 20,220 individuals by utilizing the Mental Health Inventory (MHI-5) to ask participants how often they felt nervous, depressed, calm/peaceful, down, or blue, and happy (Richard et al., 2015). Data was also collected on fruit and vegetable intake with questions designed to help participants understand portion sizes. Researchers defined adherence to fruit and vegetable intake as having five combined servings of fruits and vegetables a day. It was found that five daily servings of fruits and vegetables was associated with lower reports of moderate-to-high distress. Consuming three or more servings of vegetables daily was associated with lower reports of high distress compared to moderate distress, while two or more portions of fruits were equally associated with moderate and high distress. This suggests that vegetable intake had a greater impact on perceived distressed compared to fruit. Gibson-Smith et al. (2020) identifies that increased vegetable consumption is associated with lower reports of depression supporting Richard et al. (2015) findings. Richard et al. (2015) discussed that increased fruit and vegetable

consumption may have been linked to lower distress due to the increased amount of daily antioxidants being consumed. The researchers discuss how the main effects of antioxidants are to decrease oxidative stress in the body and to reduce inflammation which has been observed within studies working to improve overall diet quality (Bellikci-koyu et al. 2019; Kopf et al. 2018; Uemura et al. 2019; and Vanegas et al. 2017).

Bishwajit et al, (2017) studied the association between depression and the frequency of fruit and vegetable consumption in 14,133 adults throughout Bangladesh, India, and Nepal. Depression was ranked using a Self-Reported Depression screen, which evaluated participant depression symptoms over both the previous thirty days and the previous year. To assess fruit and vegetable intake, participants were asked their average servings of fruits or vegetables, with answers categorized into less than five servings daily, five servings daily, or greater than five servings daily. Overall, the countries had very low intake of fruits and vegetables with forty-one percent of adults consuming less than five combined fruit and vegetable servings. Depression scores were higher in individuals who were younger, unmarried, unemployed, without formal education, female, smoking tobacco, or drinking alcohol. Cross referencing the dietary data with the depression screen, revealed that those in India who regularly consumed less than five servings of fruits and vegetables per day were fifty-one percent more likely to report depression in the past twelve months than those who consumed five servings daily. Bishwajit et al. (2017) found that eating greater than five servings of vegetables per day was associated with decreased depression symptoms in both Bangladesh and India, which agrees with findings by Richard et al. (2015). Lower vegetable intake was associated with increased participant reports indicating both moderate and severe depression within the past 30 days. Similarly, consuming less than five

servings of fruit was associated with increased depression symptoms compared to those who ate five servings of fruit daily (Bishwajit et al., 2017).

Gibson-Smith et al. (2020) conducted research on 1,634 individuals who were part of the Netherlands Study of Depression and Anxiety provided information on mental health status, lifestyle factors, biological and anthropometric measurements over nine years. Seventy-eight percent of the initial study population was diagnosed with a lifelong anxiety or depression disorder (Gibson-Smith et al., 2020). Participants were excluded if they were diagnosed with other mental health conditions outside of depression or anxiety. Mental health status was evaluated through Composite International Diagnostic Interviews. Participants were categorized based on their mental health status at the nine-year mark. Depression symptom and anxiety severity were measured using the Inventory of Depressive Symptomatology—Self Report and Beck Anxiety Inventory, respectively. At the ninth year's assessment, participants completed a food frequency questionnaire (containing 238 items) that was used to assess individual food group associations with depression and anxiety. Gibson-Smith et al. (2020) found that increased vegetable and non-refined grain intake was significantly associated with decreased severity in depression and anxiety symptoms, which agrees with the findings of Richard et al. (2015) and Bishwajit et al. (2017). Gibson-Smith et al. (2020) study population contained primarily participants that were already diagnosed with anxiety and depression. As the individuals were part of the Netherlands Study of Depression and Anxiety it is possible that they were receiving or had already completed therapeutic, pharmacologic, or psychological treatment over the course of the nine-year period, which may have altered the association between symptom severity and diet (Gibson-Smith et al. 2020).

Compared with the small sample sizes in many microbiome studies, Richard et al. (2015), Bishwajit et al. (2017), Gibson-Smith et al. (2020), and Schweren et al. (2020) studies were completed with large populations providing a better representation of the public. Difficulties with large population studies include the fact that they rely on self-reporting from the participants, which could alter the data.

Richard et al. (2015), Bishwajit et al. (2017), and Gibson-Smith et al. (2020) study results all indicate that fruit and vegetable intake is inversely associated with depression severity. However, both Richard et al. (2015) and Bishwajit et al. (2017) acknowledge the impact that mental distress and depression have on the appetite by decreasing it, which likely limits intake of all food groups. Bishwajit et al. (2017) and Richard et al. (2015) both used the general populations, similarly to Schweren et al. (2020), meaning that individuals were not excluded from the studies for having other health conditions. A more targeted research approach specifically analyzing fruit and vegetable intake, such as the design by Gibson-Smith et al. (2020), instead of analysis of the overall diet, may have contributed to the reported associations between diet and depression compared to the results that Schweren et al. (2020) received. Hiel et al. (2019), Kopf et al. (2018), and Vanegas et al. (2017) found that increased fruit and vegetable intake was associated with an overall improved microbiome and gut health. Increased whole grains, fermented dairy products, and vegetable intake was associated with decreased inflammation, which is associated with decreased stress. Karl et al. (2017) and Nishida et al (2019) found that both physiological and psychological stress were negatively associated with gut microbiome health. Schweren et al. (2020), while not researching the impacts of diet on the microbiome, found that stressful life events negatively impacted diet quality. Lower-quality diets, which are associated with increased red meats, butter, hard margarines, and sugar-

sweetened beverages, may not have a wide variety of whole grains, fruits, or vegetables, which have been associated with positive gut health. Interventions targeting an increase in fruits or vegetables may provide a short-term improvement in mental health as suggested by Schweren et al. (2020) and short-term improvements in microbiome health as reported by Hiel et al. (2019).

Dietary Interventions and Perceived Psychological Wellbeing

Kennedy et al. (2017) studied the impacts of a supplementary fortified breakfast bar on the mental health and processing capabilities of healthy adults. The randomized double-blind study had a group of forty-seven participants taking the fortified bar and a group of forty-eight taking a calorically similar unfortified bar as a placebo. Both breakfast bars were 220 calories and provided 4.7g of dietary fiber, 8 g of sugar, and 9.6 g of protein. The placebo bar had white flour, sugar, eggs, vegetable oil, and almond flavoring, while the fortified bar had whole wheat flour, various nuts, whey protein, almond and coconut flour, quinoa, ground coconut, coconut oil, quinoa, various chocolate products, and added L-theanine, lysine, vitamins B6, B9, B12, zinc gluconate, magnesium citrate and caffeine. The participants ate the breakfast bar daily for eight weeks with a battery of computerized cognitive tests being conducted prior to intake of the breakfast bar, then 40 minutes after consumption, and 160 minutes after consumption. Mood was tested using the 16 Bond-Lader visual assessment with results combined into three mood factors of alert, content, and calm (Kennedy et al., 2017). Participants also completed a Depression Anxiety and Stress Scale (Kennedy et al., 2017). The study found the fortified breakfast bar participants had significant improvements on the cognitive tests compared to those on the placebo bars. There was a significant increase in the fortified group with alertness compared to the placebo group after consumption of the breakfast bar. Kennedy et al. (2017) did not find long term improvements in between the start or end of the intervention.

The increased alertness felt by the fortified group could be contributed to the caffeine within their breakfast bar, but the researchers discussed that the fortified bar had 21.5 mg caffeine and the typical dose of caffeine is between 75-200mg. Kennedy et al. (2017) was unable to pinpoint what aspect of the fortified breakfast bar provided short term increases in mental alertness, but the researchers suggested several ideas for continued study. Despite finding significant short-term improvements, the Kennedy et al. (2017) study was completed with a small population of healthy young adults with no other chronic conditions. This population is not representative of the general public and further research is needed.

Conner et al. (2017) and Brookie et al. (2017) investigated the effect of mood with interventions performed on 171 college students with low fruit and vegetable consumption over the course of fourteen days. These research articles were published based on the same clinical trial with Conner et al. (2017) focusing on psychological impact and Brookie et al. (2017) focusing on dietary intervention. Interventions included continuation of a normal diet, receipt of a voucher to purchase own fruits and vegetables with two daily text reminders to increase fruits and vegetables (EMI), and fruits and vegetables being provided for the duration of the study (FVI). Prior to starting the intervention and at the end of the study, participants took a depression and anxiety symptom survey and provided a blood sample. Participants in the control, FVI, and EMI groups recorded their daily perceptions of positive and negative mood, energy, and flourishing via a cell phone survey questionnaire. The EMI group received text messages targeting participants' motivation as well as providing education on the benefits of eating fruits and vegetables (Brookie et al., 2017). While participants in both the voucher and provided fruits and vegetables interventions increased intake to 3.7 servings of fruit and vegetables a day (Brookie et al., 2017), the results indicated that only the FVI group had a significant increase in

perceived psychological well-being, specifically in flourishing and perceived vitality. Conner et al. (2017) indicates potential reasons for this change could be that the provided fruits and vegetables were raw, providing increased fiber and micronutrients, or that it increased participants' intrinsic motivation to increase consumption to avoid wasting the food items. Brookie et al. (2017) noted that in a post study survey, FVI participants indicated primarily eating their provided fruits and vegetables as raw snacks, while EMI participants indicated increased vegetable content in meals, which could have been from raw, frozen, or canned vegetable sources. Brookie et al. (2017) discussed that using electronic intervention increased fruit and vegetable consumption rates similarly to that of face-to-face dietary interventions, which may make it a feasible option for large-scale fruit and vegetable interventions within the target populations.

While there was a relatively small sample size, Conner et al. (2017) and Brookie et al. (2017) provided data from three interventions and was one of the first to look at how psychological wellbeing is affected with just increasing fruit and vegetable intake. Conner et al. (2017) and Brookie et al. (2017) determined that fourteen days is too short to have many long-term physical changes in the bodies' vitamin biomarkers, but the data could potentially be correlated with perceived psychological well-being by taking part in selfcare, in this case by increasing fruit and vegetable intake. Schweren et al. (2020) also discussed that perceived mental health benefits from dietary interventions could be related to viewing increased dietary quality as selfcare. As Conner et al. (2017) and Brookie et al. (2017) results indicated positive fruit and vegetable intake in inventions targeting fruit and vegetable availability and education, further research would be needed to determine impact over a longer period of time and feasibility of interventions as it is improbable to provide all individuals with fruits and vegetables indefinitely.

Jacka et al. (2017) conducted the SMILES study which analyzed the impact of dietary intervention on adults with major depressive disorder and who had poor diet quality. For a twelve-week period, Jacka et al. (2017) had thirty-three participants partake in dietary intervention while thirty-four participants were part of a social support group. Dietary intervention included seven hours of nutrition education and counseling provided by an accredited dietitian with the focus on increasing consumption of whole grains, vegetables, fruits, legumes, low-fat unsweetened dairy, raw unsalted nuts, fish, lean red meat, chicken, eggs, and olive oil while reducing refined cereals, sweets, fried foods, sugary beverages, and excessive alcohol consumption. Overall diet quality was assessed by the Dietary Screening Tool (DTS). Participants' depression symptoms were rated by various tools including the Montgomery-Asberg Depression Rating Scale (MADRS) (see appendix E), Hospital Anxiety and Depression Scale (HADS) (see appendix F), Profile of Mood States (POMS) and Clinical Global Impression-Improvement (CGI-I) Scale (Jacka et al., 2017). The dietary intervention group had significantly lower DTS scores than the control group at baseline, but the twelve weeks of intervention significantly improved their consumption in whole grains, fruits, dairy, olive oil, legumes, and fish (Jacka et al., 2017). The dietary intervention group had ten participants qualify for remission criteria based on their MADRS scores compared to two participants in the control group. The dietary intervention group had significant improvements in their HADS scores and had significantly lower CGI-I scores than the control group, indicating that the dietary intervention group had greater improvement (Jacka et al., 2017).

The Jacka et al. (2017) study was conducted with a small sample size, which makes it difficult to generalize the findings and could alter the significance of the findings. Despite a small sample size, Jacka et al. (2017) reported that the 2014-2015 Australian Health Survey

indicated that the majority of adults in Australia had poor fruit and vegetable intake indicating adult individuals with depression and poor diet quality likely are not a small portion of the population. Due to the nature to the interventions, there was limited blinding that could be done for the participants of the study; however, it was designed to limit participant interaction with the research staff. Jacka et al. (2017) also notes that while the study results indicated that dietary intervention could be beneficial for those with clinically diagnosed depression, there was difficultly recruiting individuals due to lack of motivation and fatigue. They indicated that future studies should attempt to utilize other technological methods to provide dietary education in order to minimize barriers to participating in the research studies.

After the seeing the successful results from Jacka et al. (2017), Francis et al. (2019) conducted a similar three-week dietary intervention with seventy-eight young adults who were diagnosed with depression or anxiety to further research the impact of dietary interventions on mental health. Participants were included if receiving the same antidepressant or psychotherapy that was not started or changed two weeks prior to starting the interventions. The dietary intervention group received a thirteen-minute educational video on dietary recommendations to reduce risk of depression. The dietary education suggested an increased intake of vegetables, fruits, whole grains, protein sources like eggs, poultry, tofu, legumes, and lean meat, unsweetened dairy, fish, nuts and seeds, olive oil, turmeric, and cinnamon (Francis et al., 2019). Participants were encouraged to decrease their consumption of refined grains, sugar, and processed meats, and were provided some food items to promote dietary compliance. Depressive symptoms were assessed by use of the Centre for Epidemiological Studies Depression Scale-Revised (CEDS-R) and the Depression Anxiety Stress Scale-21 (DASS-21), POMS to assess mood, and New General Self-Efficacy Scale (GSES) to assess self-efficacy at

baseline and once intervention ended (Francis et al., 2019). Final CESD-R assessment scores indicated significant improvement in the dietary intervention group that was evaluated to be in the clinically insignificant range compared to being elevated at baseline. There was also significant decline in DASS-21 depression and anxiety scores for the dietary intervention group compared to the control group; score decline was maintained in the three-month follow up after intervention ceased (Francis et al., 2019). The dietary intervention group also had significant increases in consuming recommended food groups that persisted after the intervention at the three-month follow up.

Francis et al. (2019) found that young adults with depression were able to increase diet quality over the intervention period by either increasing consumption of recommended food groups or decreasing processed food items. Despite perceived barriers for individuals with depression following dietary advice that were described in Jacka et al. (2017), participants did well with education video, paper resources, and limited phone support. Compared to regular visits with a Registered Dietitian in Jacka et al. (2017), Francis et al. (2019)'s interventions were cost effective and could be provided to a wider population more easily.

The fact that the participants were all undergraduate university students was a weakness for Francis et al. (2019) study; it is possible that the participants being currently enrolled in higher education at the time of the study changed their compliance to dietary recommendations compared to the general public. Francis et al. (2019) discussed having the control group consume their habitual diet, indicating that it would have been unethical to ask participants to eat lower quality diet. Further research should be conducted with a larger population group of individuals diagnosed with anxiety and depression to better generalize the recommendation of dietary

change to decrease depression or anxiety symptoms as only Jacka et al. (2017) and Francis et al. (2019) have conducted studies that analyze diet change and anxiety and depression symptoms.

Both Jacka et al. (2017) and Francis et al. (2019) had similar study designs that produced significant reductions in evaluated depression symptoms within their respective populations. Common themes of the dietary interventions included increasing whole grains, fruits, vegetables, lean proteins, fish, and olive oil, which were also used as dietary interventions within the microbiome studies (Hiel et al., 2019; Kopf et al., 2018; Uemura et al., 2019; Vanegas et al., 2017). Conner et al. (2017) and Brookie et al. (2017) increased fruit and vegetable intake within their target population, but only saw psychological benefits in the FVI intervention group that was provided the fruits and vegetables by the study. While Conner et al. (2017) and Francis et al. (2019) had similar study lengths and population ages, Francis et al. (2019) had increased improvement in depressive and anxiety symptoms while Conner et al. (2017) did not find any significant changes in anxiety or depression within the three study groups. Possible reasons for the increased symptom improvement may have been that the Francis et al. (2019) population included individuals diagnosed with depression and anxiety, or that the whole diet intervention approach with education was provided by a registered dietitian who is also able to provide optimal support for dietary compliance through dietary planning and counseling, compared to no education provided by Conner et al. (2017).

Dietary intervention, mental health, and microbiome

Jacka et al. (2017), Francis et al. (2019), and Uemura et al. (2019) all had significant increases in dietary compliance with eating food groups associated with overall health. Study designs for these three researchers all included registered dietitian-provided interventions with nutrition education and counseling. Increased consumption of whole grains and vegetables were

associated with decreased inflammation markers in Karl et al. (2017), Vanegas et al. (2017), and Kopf et al. (2018), while Borgo et al. (2019) found anorexia nervosa, and arguable poor physical health and diet quality, were associated with increased inflammatory bacteria present in the microbiome. Jacka et al. (2017) and Francis et al. (2019) found that dietary intervention on individuals diagnosed with depression or anxiety had improved symptom scores on various psychological measures in tandem with their ongoing pharmacological or psychotherapy interventions compared to the control groups. Despite Richard et al. (2015), Bishwajit et al. (2017), and Gibson-Smith et al. (2020) all finding data associating poor intake of fruits and vegetables with increased self-reported rates or diagnoses of depression, there have been few studies looking at the connection between mental health, dietary interventions, and the microbiome, indicating the need for further research to be conducted within this area.

Individuals with physical and psychological stress, depression, bipolar disorder, and anorexia nervosa were all found to have altered microbiome composition compared to control groups or baseline data (Borgo et al., 2019; Jiang et al., 2020; Karl et al., 2017; Nishida et al., 2019; Severance et al., 2017). Throughout this literature review, only one study was found that examines the impact of dietary intervention with probiotics on patients with major depressive disorder and the microbiome; however, this study is still ongoing and does not have results available at this time (Karakula-Juchnowicz et al. 2019).

Research Methodology

Given that there are a limited number of studies that examined the impacts of dietary interventions on mental health and the microbiome, the proposed study follows a design similar to that of the Jacka et al. (2017) SMILES trial with the addition of stool samples being collected to analyze the microbiome. The study would follow a shorter time period of six weeks and use

electronic nutrition education via prerecorded videos as was done in Francis et al. (2019) in conjunction with follow up sessions with a registered dietitian. The modified Mediterranean diet recommendations of Jacka et al. (2017) included the servings of food groups listed in Table 2.

Table 2

Modified Mediterranean Diet Food Group Recommendations

Food Group	**Servings**
Whole Grains	5-8 per day
Fruit	3 per day
Vegetables	6 per day
Legumes	3-4 per day
Low-fat Unsweetened Dairy products	2-3 per day
Raw and Unsalted Nuts	1 per day
Fish	At least 2 per week
Lean Red Meats	3-4 per week
Chicken	2-3 per week
Eggs	Up to 6 servings
Olive Oil	3 tablespoons per day

Note: Dietary recommendations are from Jacka et al. (2017)

Increasing servings of various high fiber food groups would, in theory, promote increased populations of bacteria that are associated with colon health (Karl et al., 2017; Kopf et al., 2018; and Vanegas et al.,2017) and decrease reported depression symptoms as reported in Gibson-Smith et al. (2020), Richard et al. (2015), and Bishwajit et al. (2017).

Changes to dietary education are being suggested to help the study be more feasible with a larger population. An educational video that participants could replay as needed is more time- and cost effective than multiple visits with a registered dietitian. However, it would be recommended that participants get the opportunity to discuss follow up questions with a dietitian either by phone, in person, or via telehealth every two weeks throughout the study. This would allow for participants to seek guidance if needed during the study.

In order to analyze the microbiome, stool samples would be collected at baseline, three weeks, and six weeks after the study was completed. Samples would be collected and analyzed in three-week increments, as results from Vanegas et al. (2017) and Hiel et al. (2019) indicate that it takes approximately two or three weeks for dietary changes to be reflected in the microbiome. Compared to the other microbiome studies that only had samples at baseline and the completion of the study, having three samples would allow researchers to observe if there is a difference between weeks three or six within microbiome connection.

Increasing the length of the study compared to Francis et al. (2019) would potentially allow for two changes in the microbiome bacteria population as participants either increase or decrease dietary compliance. There is also the secondary gain of having a longer intervention period to evaluate the changes in psychological scores for anxiety or depression, which could be compared to the three-week intervention of Francis et al. (2019) or the twelve-week intervention of Jacka et al. (2017).

Summary

As Schweren et al. (2020) discussed, diet quality is likely not a predictor of development of mental health disorders, but various studies discussed in the literature review indicate that diet can have an impact on the participants' perceived mental state and may also impact the results psychological testing measures for anxiety and depression. Dietary intervention may be perceived as acts of self-care that temporarily improve gut microbiota as shown in Hiel et al. (2019). Dietary interventions should be studied to determine their impact on mental health and to compare microbiome and symptom changes to control groups, baseline data, or other forms of psychological or therapeutic interventions. In the following chapter the methodology of the proposed research study is explained in greater detail. The proposed study will combine aspects

of Jacka et al. (2017) modified Mediterranean diet interventions with analyses of the microbiome at three separate points to further to explore connections between the microbiome, mental health, and dietary interventions.

Chapter 3 Methodology

This chapter will describe a potential study design to examine the how dietary interventions impact the microbiome's bacterial populations and the symptom severity of anxiety and depression. There is limited research on the impact of improving the gut microbiota via dietary intervention and how it may impact mental health. Topics addressed in this chapter include research design, data analysis plan, threats to validity, and ethical procedures, followed by a summary of the reviewed topics.

Research Design

Research Question:

To what extent does altering the microbiome in individuals diagnosed with major depressive disorder or anxiety through, improved diet quality, impact symptom severity on anxiety and depression assessment scores?

Hypotheses:

H_o: Altering the microbiome in individuals diagnosed with MDD or anxiety, through improved diet quality, does not impact symptom severity on anxiety and depression assessment scores.

H_a: Altering the microbiome in individuals diagnosed with MDD or anxiety, through improved diet quality, reduces symptom severity on anxiety and depression assessment scores.

Sub Question 1:

Does improving diet quality in individuals diagnosed with MDD or anxiety, by following a modified Mediterranean diet, alter microbiome bacterial populations?

Hypotheses

H_{o1}: For individuals diagnosed with MDD or anxiety, improving diet quality by following a modified Mediterranean diet does not alter microbiome bacterial populations.

H_{a1}: For individuals diagnosed with MDD or anxiety, improving diet quality by following a modified Mediterranean diet significantly increases beneficial microbiome bacterial populations.

Sub Question 2:

Does improving diet quality in individuals diagnosed with MDD or anxiety, through a modified Mediterranean diet, impact symptom severity on anxiety and depression assessment scores?

Hypotheses

H_{o2}: Improved diet quality achieved by following a modified Mediterranean diet has no effect on the severity of depression and/or anxiety symptoms in individuals diagnosed with MDD or anxiety.

H_{a2}: Improved diet quality achieved by following a modified Mediterranean diet reduces the severity of depression and or anxiety symptoms in individuals diagnosed with MDD or anxiety.

Table 3

Research Questions and Variables

Research Question	Independent	Dependent	Confounding
To what extent does altering the microbiome in individuals diagnosed with major depressive disorder or anxiety through, improved diet quality, impact symptom severity on anxiety and depression assessment scores?	Diet quality	Symptom severity of anxiety and depression assessment scores Microbiome bacterial populations	medications
Does improving diet quality in individuals diagnosed with MDD or anxiety, by following a modified Mediterranean diet, alter microbiome bacterial populations?	Diet quality	Microbiome bacterial populations	medications
Does improving diet quality in individuals diagnosed with MDD or anxiety, through a modified Mediterranean diet, impact symptom severity on anxiety and depression assessment scores?	Diet quality	Symptom severity of anxiety and depression assessment scores	medications

Study Design

The study proposed is a single blind clinical trial with an intervention period of six weeks. The participants cannot be blinded due to the dietary intervention portion of the trial. The statisticians and investigators will be blinded to limit bias. Diet quality scores will be assigned

after completing the Diet History Questionnaire III at baseline and six weeks; it will evaluate dietary intake over the previous month. Participants will complete three twenty-four-hour recalls (see appendix E) at the start of the third week of the trial. Diet quality scores will be assigned related to compliance with the recommendations of the Modified Mediterranean diet. In addition to diet quality, participants will be assessed based on psychological screens ranking the severity of their depression and anxiety scores (see appendixes C and D). To assess for changes in the microbiome, all stool samples will be collected over a period of 72 hours prior to staring the intervention, at the start of week three, and at start of week six.

Research from Vanegas et al. (2017) and Hiel et al. (2019) indicated that dietary changes take approximately two or three weeks to impact the microbiome. Thus, a study length of six weeks is being proposed to allow time for the microbiome to change with dietary intervention, as well as to further investigate whether an intervention in between the time lengths of Francis et al (2019) and Jacka et al (2017) provides similar results. Evaluating bacterial populations in the microbiome at three points will allow researchers to analyze potential trends over a period of time that should be long enough to allow for one or potentially two microbiome changes, depending on ongoing dietary compliance.

Setting

The study will take place in Madison Wisconsin at the Rogers Behavioral Health Clinic. Adults participating in either the Depression and Anxiety Recovery or the OCD and Anxiety IOP programs will be eligible for the study. IOP programming is offered five times a week with three-hour sessions. Individual treatment plans will vary between participants but will encompass cognitive behavioral therapy (CBT), exposure and response prevention (ERP), dialectical behavioral therapy (DBT), behavioral activation (BA), experiential therapy, medication

management, and therapy offered in individual, group, and family sessions. An IOP program is being chosen over a Partial Hospitalization Program (PHP) due to the fact that increased time in PHP would include meals being provided by the facility which could alter dietary compliance if the meals served did not align with the modified Mediterranean diet.

Sample population

Individuals will meet inclusion criteria if they are over eighteen years of age and have a diagnosis of major depressive disorder or an anxiety disorder meeting the (DSM-V) criteria (see appendix A and B). Participants must be enrolled in an IOP program in the Rogers Behavioral Health Madison Clinic and receiving psychological treatment. Participants must be receiving the same treatment regimen two weeks prior to the start of the intervention and throughout the study.

Exclusion criteria include individuals who are pregnant or have history of eating disorders or metabolic disease(s) including phenylketonuria, fructose intolerance, galactosemia, or maple sugar urine disease. Participants will be excluded if they have medical conditions that could be adversely affected by diet change such as diabetes, kidney disease, cancer, severe food allergies, Celiac disease, or have a history of psychological illness other than depression or anxiety disorders.

Recruitment

The method to recruit participants will be based upon enrollment within the Madison IOP. Candidates will be offered an opportunity to participate in the trial when entering treatment. If participants meet inclusion and exclusion criteria, they will be provided a financial incentive in the form of a Visa® gift card of fifty dollars to help offset any additional cost of groceries pertaining to dietary recommendations. The financial incentive will be provided to all participants. Recruitment will continue until meeting the minimum of the sample size. This

means that individuals may be in different phases of the clinical trial at any given time in the study.

Sample Size

A sample size of 109 participants must be obtained to maintain a 5% margin of error and a 95% confidence interval (Raosoft, 2004). The sample size was calculated through the use of the Raosoft sample size calculator and is based upon a population size of 150, with 64 individuals will be recruited to each the dietary intervention and habitual diet group account for 15% attrition as used in Jacka et al (2017).

Data Collection Plan

Data will be collected at three separate points during this intervention. The first set of data will be collected at the start of the study which will be referred to as baseline for analysis of the results. The next set of data will be collected at the beginning of week three, which will be halfway through the intervention period. The final data collection point will be at the six-week mark which will indicate the end point of the study. At week one and week six participants will have their height and weight collected to calculate BMI. Demographic data including age, gender and race will be collected from the online electronic medical record.

Dietary compliance will be assessed through use of the three twenty-four-hour recalls (appendix G) collected at the third week of the trial as well as assessment of the Diet History Questionnaire III (DHQ III) at the end of the study. DHQ III will be completed utilizing a computer due to the nature of the participants' answers for certain food items triggering embedded questions to provide additional details (National Cancer Institute Division of Cancer Control & Population Sciences, 2020). From the DHQ III and dietary recalls dietary compliance

will be ranked using the Mediterranean Diet Adherence Screener (MEDAS) (Guerrero & Pérez-Rodríguez, 2017) (see appendix H).

Stool samples will be collected prior to starting the intervention, at the start of week three, and at the start of week six for a period of seventy-two hours using the REAL Stool Sample Collection Kit (Real Laboratory SL, 2021). Collection and analysis of stool samples are further discussed in the instrumentation section.

Dietary Intervention

Individuals selected for the dietary intervention group will receive an educational video, created by the study dietitian on the modified Mediterranean diet. This video will serve as a tool to assist participants with questions they may have between visits with the registered dietitian. Initial dietary education will be based on the modified Mediterranean diet recommendations and will provide recipes (see appendix I) and meal plan ideas to the individuals. The modified Mediterranean diet encourages increased consumption of whole grains, fruits, vegetables, legumes, lean meats and poultry, low fat unsweetened dairy, and olive oil. Table 2 lists the specific amounts recommended for each food group that will be used to rank dietary compliance. Individuals in the habitual diet (control) group will receive general education regarding nutritional intake based on MyPlate guidelines and recommendations.

The educational video can be rewatched as needed to answer basic education questions. In addition to the initial dietary education video, participants in the dietary intervention group will offered an opportunity to meet with a dietitian every two weeks for a total of three meetings where participants can ask questions and problem solve concerns with dietary compliance. Follow-up meetings with the dietitian will take place either in person, by telephone or by

telehealth depending on patient preference. The habitual diet control group will not be offered follow up visits with a dietitian.

Individuals in both the dietary intervention and habitual diet group will be provided a $50 Visa® gift card at the start of their intervention to use at their grocery store of choice to supplement additional costs to following a modified Mediterranean diet or act as an incentive to not drop out of the study depending on which group participants are placed in.

Instrumentation

Diet quality will be assessed at 3 separate points. At baseline and six weeks, all participants will complete the Diet History Questionnaire III, which will evaluate dietary intake over the past month (National Cancer Institute Division of Cancer Control & Population Sciences, 2020). The Diet History Questionnaire III will be given to participants in the first week of their intervention to gather data on dietary habits the month prior; this questionnaire will be given to participants during week six of their intervention to complete again. Upon completion, a dietary quality score will be assigned (with scores ranging from zero to thirteen based on compliance with dietary recommendations for the modified Mediterranean diet following the Mediterranean Diet Adherence Screener (MEDAS) (Guerrero & Pérez-Rodríguez, 2017) (see appendix F). If an individual scores eight or above, they will be marked as being compliant. Scores of seven or below will be marked as non-compliant. The modified Mediterranean diet does recommend alcohol intake; however, as alcohol may negatively interact with medications, it has been removed from the study's dietary recommendations and from the evaluation form for complying with the Mediterranean diet. A higher score indicates increased compliance with following the Mediterranean diet guidelines. At week three, participants will complete three 24-

hour dietary recalls (see appendix G), which will be evaluated by the dietitians and used for the week four follow-up appointment to answer participant questions.

The proposed depression and anxiety assessments include the Montgomery–Asberg Depression Rating Scale (MADRS) and Hospital Anxiety and Depression Scale (HADS) (see appendixes E and F), which will be collected at baseline, week three, and week six. These scales are being used as they were the same assessments utilized in Jacka et al (2017) and Francis et al. (2019).

Participants will collect all stool samples produced over a seventy two-hour period at baseline, week three and week six where samples will be collected using a REAL Stool Sample Collection Kit. A service will be contracted to allow for the stool samples to be collected over the 72-hour period without participants needing to deliver samples to the UW Madison Biotechnology Center. Samples will be stored and frozen at -80^{o}C and when all samples have been collected for the designated time, a 16S DNA sequence will be run on the stool samples (Fischer, Zeller, & Hyman, 2021). A 16S sequence will provide data on the rRNA genes in bacteria within the V3-V4 hypervariable regions; this will allow for identification of bacterial species down to strain level identification within the stool samples (University of Wisconsin Madison, 2021).

Specific bacteria that will be assessed are the bacteria that have been identified and discussed throughout the literature review as shown in Table 1. The bacteria will be assessed by completing ordination and cluster analyses on the operational taxonomic unit (OUT) identified in the samples. A differential analysis will be conducted at the phylum and genus level to determine relative abundances for the samples.

Data analysis plan

Descriptive statistics of gender, age, and demographics will be analyzed using a t-test to see if there is a significant difference between the habitual diet and dietary intervention group. A t-test will be used to test for significant differences in age, as well as BMI changes over the course of the intervention.

Changes in the psychiatric scores and diet scores will be assessed using a mixed effect model repeated measure (MMRM). MMRM is the preferred method of analyzing clinical data in psychiatry as it allows for all participant data to be utilized even if individuals did not complete the trial. All individuals going through therapeutic treatment would be expected to have a reduction in severity of anxiety and depression scores.

Analysis of the diet quality and psychological measures at baseline, week three, and week six between participants will be completed using a paired t-test for continuous measures, which was the statistical method for Francis et al (2019). Separate T-tests will be run for MADRS score changes and HADS score changes to determine change over the course of the intervention period. In order to compare results between the dietary intervention and the habitual diet group, and independent t-test will be run with diet quality score as the independent variable and symptom severity of the MADRS or HADS score as the dependent variable with separate T tests being run for each diagnosis.

Paired T tests will be run for the microbiome bacterial populations identified in the 16s DNA analysis. The data will be compared for populations at baseline, week three and week six of the intervention to determine if a change in the microbiome has taken place.

The statistical test used for the main research questions will be a MANOVA test with dietary compliance, categorized as compliant or non-compliant, as the independent variable. The

dependent variables will be the microbiome populations, numerical, and the HADS and MADRS scores, numerical. Tests will be run separately for both HADS and MADRS scores.

Inferential Statistics

Table 4

Inferential Statistics

Research Question	Independent Variable?	Potential Responses	Level of Measurement	Dependent Variable?	Potential Responses	Level of Measurement	Test of Significance
Main	Diet quality	Compliant Non-compliant	categorical	Symptom Severity of MDD and GAD assessments Altered microbiome populations	0-60 Or 0-21 Continuous	numerical	MANOVA
Sub 1	Diet Quality	0-13	numerical	Microbiome populations	continuous	numerical	paired T test
Sub 2	Diet Quality	0-13	numerical	Symptom Severity of MDD and GAD assessments Week 6	0-60 or 0-21	numerical	Paired T test

Randomization and Blinding

The randomization of participants will be computer-generated by a research assistant who will not be involved in the recruitment process or conducting assessments. Each participant will be assigned a computer generated 12-digit identification number for all assessments data to be tracked with. The research assistant that randomly generates the identification number will have the participants assigned Rogers Behavioral Health identification number to allow for staff working at Rogers to provide information without using the patients first or last name.

Group assignments will be given to the dietitian. Once baseline assessments have been completed, the participants will be informed of their group assignments and those in the dietary intervention group will be provided with education materials (modified Mediterranean diet recipes, see appendix I, and the pre-recorded educational video). Participants will be prompted to not discuss diet with existing treatment team members (psychologist, therapist, nurse practitioner) throughout the study. Due to needing to distribute education materials, the dietitian will need to be aware of the group assignments.

In an attempt to reduce bias, participants will be given the symptom severity questionnaires to fill out by the participants attending psychologist; the psychologist will not know what group the participant has been assigned. Completed MADRS and HADS scores will be provided to the research assistant with the participants Rogers assigned identification number. The data will be entered under the participants randomly generated identification code. The Diet History Questionnaire III will be filled out electronically on a laptop by the participant. Completed data will be sent to the dietitian to calculate diet score based on the responses how they comply with the modified Mediterranean dietary recommendations.

Threats to validity

Treats to validity include participant error in stool sample collection, which would present with less accurate microbiome data. Another threat to validity is utilizing three assessments for collecting symptom severity and dietary history. Using several assessments may cause survey fatigue for the participants and may result in answers not being accurate reflections due to intent to complete the survey quickly.

The three-day 24-hour diet recall collected at week three may also be a potential cause of a threat to internal validity. Food records may cause participants to either over or underestimate

consume amounts. The process of writing down dietary intake for 24 hours may result in increased compliance with the modified Mediterranean diet during that time frame due to increased awareness of what is being consumed.

Throughout the trial, individuals will be continuing with therapeutic treatment for their anxiety and or depression. It is plausible that overall improved depression and anxiety symptoms will result in improved dietary quality in both the habitual diet and dietary intervention groups. Previous studies have indicated that low dietary quality in individuals with depression could be related to low motivation and altered dietary patterns as a result (Bishwajit et al., 2017; Richard et al. 2015). Improvement of these symptoms could result in normalization of regular eating and increased motivation to prepare food items reducing reliance on convenience products.

Ethical Procedures

An Institutional Review Board application will be completed prior to starting the recruitment process for the trial. Upon recruitment, participants will be provided an informed consent document that will be reviewed with the participant by a researcher

To protect the identity of participants and prevent bias in the data analysis, participants will be assigned a randomly generated twelve-digit number to identify and keep track of the progression of microbiome, dietary compliance, and symptom severity scores for anxiety and depression. To prevent unintentional bias, a research assistant that was not part of the recruitment process and did not interact with participants will enter data, which will then be analyzed by a different research assistant.

The research data will be stored on a password protected external hard drive that will be stored in an office in the Madison Rogers PHP building. Storing data on site will allow for better access to participants for retrieving data as participants will be at IOP programming five days a

week. Data will be kept for 12 months after the completion of the study to allow for additional analysis of data if desired.

Summary

This proposed study will focus on testing connections between dietary intervention, mental health, and the microbiome. Over the course of six weeks, participants will follow the modified Mediterranean diet or their habitual diet while providing stool samples at the beginning of the study and beginning of week three and week six. Participants will continue in their normal therapeutic treatment within the Rogers Behavior Health Madison Clinic and will take the MADRS and HADS assessments to rate changes in severity of depression and anxiety symptoms. Data will be analyzed to determine if changes in eating habits, microbiome composition, and symptom severity occurred during the timeframe pre-intervention to post intervention. Data will also be analyzed between the dietary intervention and habitual diet groups to determine if there are significant differences between both groups.

This study will be shorter in length compared to the SMILES study by Jacka et al. (2017) and longer than the intervention period of Francis et al. (2019) in order to determine if intervention length has an impact on study results. The effects of dietary intervention on microbiome and psychiatric assessments will also be analyzed as well as potential connections between the microbiome populations and mental health. Chapters 4 and 5 will discussed the anticipated results and include a discussion with concluding statements on the topic respectively.

Chapter 4 Anticipated Results

Characterization of study Participants

At total of 150 participants will be recruited to participate in the study with 75 individuals being randomized into the dietary intervention and 75 into the habitual diet group. The dietary intervention group will receive education about the modified Mediterranean diet via sessions with a dietitian and a pre-recorded educational video, while the habitual diet group will only have one session with the dietitian reviewing MyPlate eating guidelines. It is estimated that 23 participants will drop out due to the attrition rate observed by Jacka et al. (2017). Likely causes for not completing the trial include early discharge from the Madison IOP or choosing to not follow through on the intervention. Figure 1 shows how many study participants are anticipated to complete the trial.

Figure 1

Flowchart illustration for the study participants

Baseline Characteristics of Study Population

Anticipated baseline characteristics of both groups are presented in Table 5. It is anticipated that there will be no significant differences in baseline characteristics between groups.

Table 5

Baseline Characteristics of dietary intervention or habitual diet groups

		Total (n=127)	DI (n=64)	HD (n=63)	P value
Demographic					
Gender % female	%(n)	55(70)	58(38)	52 (32)	0.72
Age	M(SD)	30.4 (6.9)	30.1 (6.8)	30.6 (7.1)	0.73
Health Measures					
BMI	M(SD)	26.8 (5.1)	26.9 (5.3)	26.6 (4.9)	0.74
Diet Quality					
MEDAS (0-12)	M(SD)	5.3 (3.1)	5.2 (2.1)	5.3 (2.1)	0.80
Psychological Measures					
MADRS (0-60)	M(SD)	26.4 (2.9)	26.3 (2.9)	26.5 (2.9)	0.77
HADS-D (0-21)	M(SD)	8.6 (1.3)	8.6 (1.2)	8.7 (1.3)	0.60
HADS-A (0-21)	M(SD)	11.3 (1.6)	11.3 (1.6)	11.4 (1.6)	0.77

Note: *BMI* body mass index, *MEDAS* Mediterranean Diet Adherence Screener, *MADRS* Montgomery-Asberg Depression Rating Scale, *HADS* Hospital Anxiety and Depression Scale

*p<0.5 indicates statistical significance

MEDAS scores and change in Psychological Tests

Dietary compliance with the modified Mediterranean diet will be collected based on the DHQIII data that will be completed by participants at baseline and week six of the study. The study's registered dietitian(s) will assign a MEDAS score based on dietary intake in compliance with the modified Mediterranean diets recommendations.

Measurements of symptom severity will be done through MADRS and HADS tests. MADRS and HADS, both anxiety and depression, will be collected at baseline, week three and

week six. HADS-Anxiety (HADS-A) and HADS-Depression (HADS-D) will be listed separately. Anticipated changes in the psychological tests are show in Table 6 from baseline to week six at the end of the intervention period. It is anticipated that both groups will experience significant decreases in MADRS and HADS scores due to participating in therapeutic treatment regardless of their assigned intervention group. However, it is anticipated that when comparing week six data for the groups, there will be a significant difference between the dietary intervention and the habitual diet group for MADRS and HADS-A scores.

Table 6

Change in Psychological symptom and MEDAS scores

Test Score	Dietary Intervention			Habitual Diet			Change between groups at 6 weeks p-value
	Baseline M(SD)	6 weeks M(SD)	p-value	Baseline M(SD)	6 weeks M(SD)	p-value	
Symptom Severity							
MADRS (0-60)	26.3(2.9)	17.4(3.7)	<0.001	26.5(2.9)	19.5(2.3)	<0.001	<0.001
HADS-D (0-21)	8.6(1.2)	7.1(1.1)	<0.001	8.7(1.3)	8.3(1.4)	<0.001	0.035
HADS-A (0-21)	11.3(1.6)	8.7(1.3)	<0.001	11.4(1.6)	10.3(1.6)	<0.001	<0.001
Diet							
MEDAS Score	5.2(2.1)	7.9(1.4)	<0.001	5.3(2.1)	7.0 (1.4)	<0.001	<0.001

Note: p-value < 0.05 is considered significant.

Changes in microbiome from baseline, week three, and week six

Microbiome data will be collected at baseline, week three, and week six over a 72-hour period. Data will be collected via stool samples and analyzed by UW Madison Biotechnology Center via a 16S DNA test. The microbiome populations listed for data analysis will be the most prevalent strains found in the participants samples. Anticipated results for the change in

microbiomes bacterial populations are shown in Table 7 with data from baseline, week three and week six. It is anticipated that the *Bifidobacterium, Lachnospiraceae,* and *Ruminococcus* bacterial populations will significantly increase in the dietary intervention group and remain relatively unchanged in the dietary interventions group. It is anticipated that changes noted in the dietary intervention group will be significantly different from those in the habitual diet group.

Table 7

Microbiome Population Percentages at baseline, week three, and week six

	Dietary Intervention				Habitual Diet				Change between Groups at Week 6 P Value
Bacteria	Baseline	Week 3	Week 6	Change from Baseline to Week 6 P Value	Baseline	Week 3	Week 6	Change from Baseline to Week 6 P Value	
Bifidobacterium % pop (SD)	9.8(1.3)	10.9(1.2)	11.9(1.1)	<0.001	9.8(1.2)	11.2(1.1)	10.9(1.1)	<0.001	<0.001
Proteobacteria % pop (SD)	0.9(0.5)	1.1(0.6)	0.8(0.5)	0.32	0.9(0.6)	0.9(0.6)	1.0(0.6)	0.50	0.68
Streptococcus % pop (SD)	1.1(0.6)	1.0(0.6)	0.7(0.5)	0.003	1.1(0.6)	0.9(0.6)	1.0(0.6)	0.53	0.01
Lachnospiraceae % pop (SD)	0.9(0.6)	1.9(0.6)	2.4(0.6)	<0.001	1.0(0.6)	1.0(0.6)	1.0(0.6)	0.16	<0.001
Ruminococcus % pop (SD)	1.0(0.6)	1.9(0.6)	2.4(0.6)	<0.001	1.0(0.6)	1.0(0.5)	1.0(0.5)	0.002	<0.001

Note: p-value < 0.05 is considered significant.

Comparison of dietary compliance and the impact on psychologic scores and microbiome populations

A MANOVA statistical test will be conducted on the results to compare the significance between dietary compliance with the mental health assessment scores and microbiome population changes. The independent variable will be dietary compliance marked as compliant or non-compliant (MEDAS score >8) with the dependent variables being the scores at week six for the MARDS, HADS-A, HADS-D, and microbiome populations. MANOVA tests will be conducted with data representing the change from baseline to week six of the intervention period. Table 8 shows anticipated descriptive statistics for the MANOVA results with a breakdown of dependent variables based on dietary compliance. Table 9 shows the anticipated results for the tests of Between-Subjects analyzing the variation between the dependent variables.

Table 8

MANOVA Descriptive Statistics

	Dietary Compliance	Mean	Std. Deviation	N
MADRS Scores	compliant	18.75	3.605	68
	non-compliant	18.10	2.796	59
	Total	18.45	3.258	127
HADS-A Scores	compliant	9.01	1.501	68
	non-compliant	9.56	1.600	59
	Total	9.27	1.566	127
HADS-D Scores	compliant	7.24	1.283	68
	non-compliant	7.42	1.367	59
	Total	7.32	1.321	127
Bifidobacterium Population %	compliant	11.49	1.174	68
	non-compliant	11.24	1.283	59
	Total	11.38	1.227	127
Proteobacteria Population %	compliant	.82	.472	68
	non-compliant	.84	.468	59
	Total	.83	.469	127
Streptococcus Population %	compliant	.84	.507	68
	non-compliant	.90	.590	59
	Total	.87	.546	127
Lachnospiraceae Population %	compliant	1.75	.972	68
	non-compliant	1.53	.960	59
	Total	1.65	.969	127
Ruminococcus Population %	compliant	1.62	1.084	68
	non-compliant	1.61	.960	59
	Total	1.61	1.024	127

Table 9

Test of Between-Subject Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	MADRS	13.278[a]	1	13.28	1.253	.265	.010
	HADS-A	9.37[b]	1	9.37	3.910	.050	.030
	HADS-D	1.12[c]	1	1.12	.641	.425	.005
	Bifidobacterium	2.00[d]	1	2.00	1.333	.251	.011
	Proteobacteria	.02[e]	1	.02	.098	.755	.001
	Streptococcus	.14[f]	1	.14	.465	.496	.004
	Lachnospiraceae	1.55[g]	1	1.55	1.656	.201	.013
	Ruminococcus	.003[h]	1	.003	.003	.960	.000
Intercept	MADRS	42901.47	1	42901.47	4049.94	<.001	.970
	HADS-A	10898.57	1	10898.58	4548.23	<.001	.973
	HADS-D	6788.40	1	6788.40	3881	<.001	.969
	Bifidobacterium	16330.67	1	16330.67	10871.9	<.001	.989
	Proteobacteria	86.90	1	86.90	392.76	<.001	.759
	Streptococcus	95.31	1	95.31	318.52	<.001	.718
	Lachnospiraceae	339.62	1	339.62	363.80	<.001	.744
	Ruminococcus	328.79	1	328.79	310.83	<.001	.713
Compliance	MADRS	13.28	1	13.28	1.25	.265	.010
	HADS-A	9.370	1	9.37	3.91	.050	.030
	HADS-D	1.12	1	1.12	.64	.425	.005
	Bifidobacterium	2.00	1	2.00	1.33	.251	.011
	Proteobacteria	.022	1	.02	.098	.755	.001
	Streptococcus	.139	1	.14	.465	.496	.004
	Lachnospiraceae	1.55	1	1.55	1.656	.201	.013
	Ruminococcus	.003	1	.003	.003	.960	.000

a. R Squared = .010 (Adjusted R Squared = .002)
b. R Squared = .030 (Adjusted R Squared = .023)
c. R Squared = .005 (Adjusted R Squared = -.003)
d. R Squared = .011 (Adjusted R Squared = .003)
e. R Squared = .001 (Adjusted R Squared = -.007)
f. R Squared = .004 (Adjusted R Squared = -.004)
g. R Squared = .013 (Adjusted R Squared = .005)
h. R Squared = .000 (Adjusted R Squared = -.008)

Note: p-value < 0.05 is considered significant.

Summary

Anticipated results were shown in tables 5-8 with indications of anticipated significant changes over the course of the six-week intervention period. Anticipated changes between groups were also discussed and noted within each section. Chapter 5 will discuss the anticipated results and how they related to the existing literature. The strengths and weaknesses of the proposed study will also be discussed, as well as anticipated needs for future research.

Chapter 5 Discussion

Mental health diagnoses do not currently have any dietary recommendations. Results from studies conducted by Jacka et al (2017) and Francis et al (2019) found that following a modified Mediterranean diet decreased anxiety and depression symptom severity in participants. Increasing whole grains, fruits and vegetables was also found to increase beneficial bacteria within the microbiome (site). This chapter will discuss the anticipated results of the proposed study and compare those results from previous studies conducted by Jacka et al. (2017), Francis et al. (2019), and (site). It will also discuss the proposed study's strengths and limitations followed by suggestions for future research.

Interpretation of Results

This six-week single blind clinical trial will evaluate the effects of following a modified Mediterranean diet on changes present in the microbiome and symptom severity of individuals diagnosed with anxiety disorders or major depressive disorder. It is anticipated that the null hypothesis will be rejected for all secondary research questions and that all alternative hypotheses will be accepted. Hypotheses for the main research questions will be discussed further in this chapter. Expected outcomes include significant decreases to symptom severity scores as reflected in MADRS, HADS-Depression, and HADS-Anxiety scores, increased dietary compliance as evident by MEDAS scores higher than eight, and increased population percentages of beneficial microbiome bacteria in the dietary intervention group compared to the habitual diet group.

Characterization of the Study Population

The study participants will include individuals over the age of eighteen years. The anticipated results were influenced by data from Rogers Behavior health indicating those that

attend PHP programs were 55% female with an average age of thirty years old. Due to a Rogers IOP site serving as the setting for this study, it is anticipated that the population will fall within a similar range. Population characteristics are anticipated to have no significant differences between the dietary intervention and habitual diet group for age, gender, BMI, diet quality, and psychological measures. Data for the psychological measures were based on the baseline characteristics of Jacka et al. (2017)'s research.

MEDAS scores and change in Psychological Tests

The dietary intervention group is anticipated to have significant changes between all of its measures from baseline to the end of the six-week intervention period. The diet intervention group average MEDAS score increased from 5.2 at baseline to 7.9 at the end of the intervention period. While the average score was not above 8, which was the marker for dietary compliance, the score revealed significant improvement in diet quality. MADRS scores decreased indicating a symptom classification change from moderate depression severity to mild depression symptoms. HADS depression scores decreased from borderline abnormal to normal over the intervention period. HADS anxiety scores decreased from abnormal to borderline abnormal. This decrease is significant and is similar to the data found in Jacka et al. (2017)'s study.

The habitual diet group is also showed significant improvement in symptom severity scores and diet quality scores. MADRS scores decreased from moderate depression symptoms to mild depression symptoms. HADS depression scores remained in the borderline abnormal range, but analysis showed significant change within participants data from baseline to week 6. HADS anxiety scores reduced from abnormal to borderline abnormal classifications.

It is anticipated that there will be significant differences between the dietary intervention group and habitual diet group in MADRS scores, HADS-A, and MEDAS scores, based on T-

tests. HADS-D score may have a smaller decrease within each trial and comparatively due to the distribution of patients from the IOP programs as two of the IOP locations target anxiety while only one has a depression specific focus. Madison's Rogers Behavioral Health clinic has more depression-focused PHP programs making it likely that someone would transfer to an IOP program with decreased depression scores. Overall, it is anticipated that participants will have a reduction in symptom severity scores due to continuing to take part in therapeutic treatment. While possible, it is unlikely that a participant will have a significant increase in anxiety or depression symptom severity scores without dropping out of the study due to moving to a higher level of care (PHP, residential, or inpatient.)

Changes in microbiome from baseline, week three, and week six

Microbiome data will be collected at three separate points throughout the study with bacterial populations being assessed at each point in time. Data will be displayed as percent population. *Bifidobacterium, Lachnospiraceae,* and *Ruminococcus* are health promoting microbiome bacteria, while *Streptococcus* and *Proteobacteria* are associated with colorectal cancer and inflammation respectively. It is anticipated that *Bifidobacterium, Lachnospiraceae,* and Ruminococcus will increase in percent population in the dietary intervention group as individuals improve dietary compliance with the modified Mediterranean diet recommendations. Proteobacteria and Streptococcus will not change within the intervention period anticipated following the results from Jiang et al (2020).

Within the habitual diet group, it is anticipated that there will a significant change for the Bifidobacterium and Ruminococcus bacterial populations due to Richard et al (2015) and Bishwajit et al (2017) noting reduced diet quality was associated with higher depression scores.

This change would likely be due to increased diet quality within the group as individuals' anxiety and depressive symptoms decrease with continued treatment in the IOP program.

Comparison of dietary compliance and the impact on psychologic scores and microbiome populations

The MANOVA test was conducted on the week six data for both the dietary intervention and habitual diets with dietary compliance (MEDAS score greater than 8) as the independent variable. The dependent variables were the MADRS, HADS-A, HADS-D, and microbiome populations. Results from the MANOVA test indicate that there was not a significant association between an individual being compliant with the modified Mediterranean diet dietary recommendations and subsequent changes within the microbiome variables. No significance was found for the MADRS or HADS-D symptom severity scores. There was a significant difference for HADS-A symptom severity scores between patients who were non-compliant vs those who were compliant with dietary interventions.

As stated earlier within the psychological test results, it is not expected for individuals to increase anxiety and depression scores without having changes to treatment, which would likely result in either a medication change or stepping up to a higher level of care such as PHP, residential or inpatient depending on symptom severity. These changes would result in the individual not completing the study. With the anticipation that psychological symptoms will improve regardless of which group the participant is in, it is to be expected that diet quality will start to improve as well, which was suggested in Jacka et al. (2017) and Francis et al. (2019).

Within the microbiome populations it is expected that there would be an overall percentage increase for *Bifidobacterium, Lachnospiraceae*, and *Ruminococcus* bacterial populations due to increasing intake of fruits, vegetables, and whole grains. It is also anticipated

that *Streptococcus* and *Proteobacteria* populations would decrease with increasing diet quality. It is possible that Rogers Behavioral Health's use of Cognitive Behavioral Therapy (CBT) with a focus on Exposure and Response Prevention (ERP) may negatively impact the microbiome due to the nature of exposures to cause anxiety or stress. It was shown in Karl et al (2017) and Nishida et al (2019) that both physiological and psychological stress negatively impact the populations of health prompting microbiome bacteria.

Comparison to other studies

The dietary intervention group are anticipated to have significant decreases in symptom severity on the MADRS and HADS similar to the results found in Jacka et al (2017) and the results of Francis et a (2019). Compared to the results of Jacka et al (2017), it is likely that the MADRS and HADS scores will have a smaller decrease due to the proposed study being a six-week intervention rather than the twelve-week intervention implemented by Jacka et al.

The dietary intervention group is expected to have a greater improvement of dietary compliance and overall diet quality as shown in both Jacka et al (2017) and Francis et al (2019), which is expected to occur within this study due to the increased nutritional education and follow up appointments with a registered dietitian. Francis et at found that significant dietary changes could occur within a three-week timeframe, so we anticipate significant changes in diet quality over the six-week intervention period.

By following the modified Mediterranean diet, it is anticipated that *Bifidobacterium* populations will increase as the participants increased consumption of vegetables as seen by Uemura et al. (2019), Vanegas et al. (2017) and Hiel et al. (2019). Increasing consumption of whole grains over refrained grain products is expected to increase populations of the *Lachnospiraceae* bacterial populations as noted in Vanegas et al (2017). Nishida et al (2019)

found that increasing intake of probiotics reduced the populations of *Streptococcus* bacteria. Unlike other microbiome and dietary intervention studies, the proposed study is not specifying a singular food group to increase rather, modifying the overall dietary pattern. This may result in greater microbiome diversity, but less significance within individual population changes.

Strengths and Limitations

A strength for this study is the structure of the nutrition education incorporating both technological education through the pre-recorded modified Mediterranean diet video as well as follow up with a registered dietitian to assist with personalized education and support as needed. Various forms of education will assist in targeting various learning styles and likely increase dietary compliance.

Working within a IOP setting is both a strength and a limitation for this study patients are benefiting from the multimodal therapeutic programs being offered. Having the therapy provided in one location can increase compliance with treatment as an individual's therapist and psychiatrics are in one location. A limitation is that Rogers programs are voluntary meaning that participants have the ability to choose what programs they attend when at IOP, which could limit therapeutic benefit in the setting.

For the microbiome the data will be collected at three points over the course of the study allowing researchers to examine changes over time to determine how the microbiome changes with treatment for anxiety and depression. A strength includes having a dietary intervention and a control group that is following their habitual diet. However, within this set up there is a limit of not having a group of individuals without mental health diagnoses with whom to compare microbiome data.

Another limitation of this is that use of participants from only one PHP clinic may reduce patient diversity. Future studies should expand the patient population to further generalize findings for individuals diagnosed with anxiety or depressive disorders. If continuing to utilize microbiome data, it would be valuable to expand the study to have four groups including individuals without mental health diagnoses and those with mental health diagnoses partaking in a dietary intervention or habitual diet. This would allow for the findings to be generalized, as well as continuing to study microbiome differences between individuals with anxiety and depression and those who do not have diagnosed mental health concerns.

Conclusion

With the continuation of COVID-19 pandemic, mental health has become an increasing issue for many Americans. Even before the COVID-19 pandemic, many individuals did not seek treatment. The proposed research study will look at the potential relationship between dietary interventions, mental health, and the microbiome by utilizing a modified Mediterranean diet over a six-week period. The use of the microbiome data will provide objective data via changes in microbiome bacterial populations throughout the intervention period and will be compared to dietary compliance and symptom severity assessments. Continuing research on the impact of dietary interventions on individuals with mental health diagnoses, further research within this area will help create dietary recommendations for these individuals to follow in addition to their pharmacological and therapeutic treatment.

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Office use only: IRB Approval #: _____

Mount Mary University

Institutional Review Board (IRB)

for the Protection of Human Subjects

Application for IRB Review

DATA COLLECTION CANNOT BEGIN

UNTIL THE IRB HAS APPROVED THIS PROJECT

I. Required Documentation - No action will be taken without these attachments.

Are the following attached to the IRB application?

Informed Consent Document	☒ Yes	Informed Consent Documents should include an explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature, and date. See Appendix. A and use the **MMU Informed Consent Template** to avoid delays in the process.
Questionnaire/Survey Instrument(s)	☒ Yes	If a survey is being administered in any written format (e.g., survey monkey, qualtrics), a copy of that survey must accompany this application. If a survey is being conducted verbally, a copy of the introductory comments and survey questions being asked must be attached to this application. If survey includes focus group questions, a complete list of the question must be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.
Verification of Human Subjects Training	☐ Yes	Copy of transcript, certificate, or other evidence that ALL members of the research team have completed the required training. .
Copy of cooperating institution's IRB approval.	☐ Yes	Not required if there is no cooperating institution. .

II. Investigator(s):

Name: Alexandra Fischer Phone:
Affiliation with Mount Mary University (e.g., faculty, student, etc.): student
Email: fischera@mtmary.edu

Signature: _Alexandra Fischer_____ Date: 10/7/2021

Name: Phone:
Affiliation with Mount Mary University:
Email:

Signature: _____ Date:

If student, list Research Advisor and complete the application. Research Advisor must provide requested information and verify.

Department:

Research Advisor's Name: Dr. Dana Scheunemann
Email: scheuned@mtmary.edu Phone:

Research Advisor: Have you completed Human Subject's Training? ☐ Yes ☐ No

Research advisor's signature indicates responsibility for student compliance with all IRB requirements.

Signature: _____ Date:
Research Advisor

III. Project Description – Required by all applicants

Instructions: Briefly describe the proposed project including the sample and methodology (e.g., human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):

Using a single blind clinical trial model, this study will explore the relationship between the microbiome and depressive and/or anxiety symptom severity before, during, and upon completion of a modified Mediterranean diet dietary intervention.

2) Relevance to practice/body of knowledge:

The proposed research study will look at the potential relationship between dietary interventions, mental health, and the microbiome. With limited research conducted on the impact of dietary interventions on individuals with mental health diagnoses, further research within this area will help create dietary recommendations for these individuals. The use of the microbiome data will provide objective data via changes in microbiome bacterial populations that are associated with dietary interventions and further the research already conducted examining the impact between the microbiome and mental health.

3) Describe the research design (e.g., subject/participant selection and assignment, design, intervention, data analysis):

The study proposed is a single blind clinical trial with an intervention period of six weeks taking place at the Rogers Behavioral Health Madison IOP clinic. The participants cannot be blinded due to the dietary intervention portion of the trial. The statisticians and investigators will be blinded to limit bias. Diet quality scores will be assigned after completing the Diet History Questionnaire III at baseline and six weeks; it will evaluate dietary intake over the previous month. Participants will complete three twenty-four-hour recalls (see appendix E) at the start of the third week of the trial. Diet quality scores will be assigned related to compliance with the recommendations of the Modified Mediterranean diet. Individuals in the dietary intervention group will received a prerecorded educational video describing the dietary requirements and will received 2 follow up appointments with a registered dietitian. The habitual diet group will receive MyPlate dietary education at the start of the intervention period. In addition to diet quality, participants will be assessed based on psychological screens ranking the severity of their depression and anxiety scores using the MADRS and HADS symptom severity screens. To assess for changes in the microbiome, all stool samples will be collected over a period of 72 hours prior to staring the intervention, at the start of week three, and at start of week six.

Individuals enrolled in the Madison IOP program will be screened to determine if they meet inclusion criteria and will be offered the opportunity to partake in the study. If participants meet inclusion criteria, they will be provided a financial incentive in the form of a Visa® gift card of fifty dollars to help offset any additional cost of groceries pertaining to dietary recommendations. The financial incentive will be provided to all participants. Recruitment will continue until meeting the minimum of the sample size. This means that individuals may be in different phases of the clinical trial at any given time in the study. The study aims to recruit 150 individuals for the study.

Data will be collected at three separate points during this intervention: baseline, week 3 and week 6. At week one and week six participants will have their height and weight collected to calculate BMI.

Demographic data including age, gender and race will be collected from the online electronic medical record. Stool samples will be collected prior to starting the intervention, at the start of week three, and at the start of week six for a period of seventy-two hours using the REAL Stool Sample Collection Kit (Real Laboratory SL, 2021). Collection and analysis of stool samples are further discussed in the instrumentation section.
Paired T tests will be run to determine changes between baseline and completion data for both the dietary and habitual diet groups with MADRS, HADS and microbiome population scores. Comparison for between dietary and habitual diet groups will be done with independent T-test. To compare how dietary compliance impacted MADRS, HADS, and microbiome populations a MANOVA test will be run with dietary compliance as the independent variable.

4) What measurement/data collection tools are being used?

Diet History Questionnaire III, MADRS and HADS symptom severity screens, MEDAS, 24-hour dietary recall
REAL Stool Sample Collection Kit (Real Laboratory SL, 2021)

IV. Additional Project Information – Required by all applicants

1) What human subjects training has the researcher completed (e.g., course work, online certification)?

2) What process is used for obtaining informed consent (attach the informed consent application)? See Appendix for consent application.

3) Does the research include special populations?

Minors under 18 years of age?	☐ Yes	☒ No
Persons legally incompetent?	☐ Yes	☒ No
Prisoners?	☐ Yes	☒ No
Pregnant women, if affected by research?	☐ Yes	☒ No
Persons institutionalized?	☐ Yes	☒ No
Persons mentally incapacitated?	☐ Yes	☒ No

4) If **YES,** describe additional precautions included in the research procedures.

5) Does the research involve any of the following procedures?

False or misleading information to subjects?	☐ Yes	☒ No
Withholds information such that their informed consent might be questioned?	☐ Yes	☒ No
Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects?	☐ Yes	☒ No

6) If **YES**, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

7) Does the research involve measurement in any of the following areas?

Sexual behaviors?	☐ Yes	☒ No
Drug use?	☐ Yes	☒ No
Illegal conduct?	☐ Yes	☒ No
Use of alcohol?	☐ Yes	☒ No

8) If **YES**, describe additional precautions included in the research procedures.

9) Are any portions of the research being conducted online?

Survey posted on a website?	☐ Yes	☒ No	If yes, assure anonymity
URL for survey includes information that could identify participants?	☐ Yes	☒ No	If yes, assure anonymity
Invitation to participate sent by email?	☐ Yes	☒ No	If yes, assure anonymity
Items use drop-down box?	☐ Yes	☒ No	If yes, assure that items allow choice of "no response"

10) If **YES**, describe additional procedures.

11) Describe the methods used to ensure confidentiality of data obtained.

Each participant will be assigned a computer generated 12-digit identification number for all assessments data to be tracked with. The research assistant that randomly generates the identification number will have the participants assigned Rogers Behavioral Health identification number to allow for staff working at Rogers to provide information without using the patients first or last name. Data will be stored on a password protected external hard drive that will be stored in an office in the Madison Rogers IOP building.

Risks and Benefits

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional, or behavioral risk.)

Participants in the dietary intervention group will be asked to change dietary patterns to be in line with the modified Mediterranean diet, but participants are able to choose what food items they consume. Changing dietary patterns may result in some gastrointestinal discomfort if participants choose to include foods they are unfamiliar with, but otherwise there should be minimal risk for participants.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)

The proposed research study will look at the potential relationship between dietary interventions, mental health, and the microbiome. With limited research conducted on the impact of dietary interventions on individuals with mental health diagnoses, further research within this area will help create dietary recommendations for these individuals and can provide another tool help with management of anxiety and depressive symptoms. The use of the microbiome data will provide objective data via changes in microbiome bacterial populations that are associated with dietary interventions and further the research already conducted examining the impact between the microbiome and mental health.

V. Is the proposed project "research" as defined by Institutional Review Board requirements? - Required by all applicants

- Research is defined as a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.
- A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information.

Does the research involve human subjects or official records about human subjects?

☒ Yes
☐ No

***If NO STOP here and SUBMIT application*.**

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:

☐ Yes
☒ No

If the YES box is CHECKED, proceed to SECTION VI.

***If the NO box is CHECKED, STOP here, and SUBMIT application*.**

Appendix B *Consent Form*

Research Participant Information and Consent Form

Mount Mary University

Title of Study: Microbiota and Mental Health: A randomized controlled trial of dietary improvement and microbiome changes for adults with major depressive disorder depression or anxiety disorders

Invitation to Participate and Purpose of the Research You are invited to participate in a research study that seeks to evaluate the impacts of following a modified Mediterranean diet impacts the microbiome and anxiety and depression symptom severity. The study will seek to determine if the intervention results in decreased anxiety and depression symptom severity and improved healthy gut bacteria populations when dietary patterns change. There will be two groups, one group will receive the intervention and the other will not receive the intervention and will be known as the habitual diet group. Participants will be asked to fill out several 24-hour food recalls logs, take several assessment tests, and collect stool samples. Individuals in the intervention group will receive dietary education on the modified Mediterranean diet and will see a registered dietitian twice during the intervention period. They will also be encouraged to incorporate some of the foods described in their daily diet. Data will be de-identified and analyzed by researchers. Participants must be 18 years of age or older.

Benefits and Risks: This research is designed to benefit the dietetics profession, by analyzing the impact of dietary intervention on symptom severity of mental health diagnoses to provide dietary recommendations similar to what is recommended for other health conditions. Although participants may not benefit personally from being in this research study, findings generated by this research may add new knowledge to the nutritional psychology dietetics

field in general. There will be $50 Visa Gift card provided as compensation. There are no known potential risks associated with participating in this study. Please address any questions or issues of concern to the researchers using the contact information provided above.

Confidentiality: All information obtained will be kept confidential by the researchers who will be the only people with access to the data. Information obtained will be stored electronically and will be password protected. Per the U.S. Office of Human Research Protections (code §46.115), all data will be destroyed 3 years after the end of data collection. Paper files will be shredded, and electronic files will be deleted. Individual participants will not be identified in any report or publication about this study.

Contact Information If you have questions about this research study, your rights as a research subject, or would like to know the outcome of the research, please contact Dana Scheunemann, 414-930-3658, scheuned@mtmary.edu and Alexandra Fischer, 414-745-2225, fischera@mtmary.edu. If you have any questions regarding your rights or privacy as a participant in this study, please contact Dr. Tammy Scheidegger, Mount Mary University Institutional Review Board Chair, 2900 North Menomonee River Parkway, Milwaukee, Wisconsin, 53222-4597, telephone (414) 930-3434 or email schediet@mtmary.edu.

Consent By signing below, you are indicating that you have read this consent form, have been given the opportunity to ask questions, and have agreed to voluntarily participate. You may withdraw from participation at any time, or refuse to answer any question herein, without penalty or loss of benefits to which other participants are entitled.

You may request a copy of this page for your records. Thank you for your participation.

Signature of participant_____ Date _____

Other Possible Elements Needed

A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant. For research involving more than minimal risk, a statement describing any compensation for injuries and contact information. (Minimal risk is a risk of harm to the participant that is no greater than the risk encountered in normal, day-to-day activities or during routine physical or psychological examinations.) If the participant is a patient or client receiving medical, psychological, counseling, or other treatment services, there should be a statement that withdrawal from the study will not jeopardize or otherwise affect any treatment or services the participant is currently receiving or may receive in the future. Participants also should be told whether their data will be destroyed should they withdraw from the study. If a survey instrument or interview questions are used and some questions deal with sensitive issues, the participants should be told they may refuse to answer individual questions.

Appendix C *DSM V MDD diagnostic criteria*

Major Depressive Disorder

Diagnostic Criteria

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

Note: Do not include symptoms that are clearly attributable to another medical condition. 1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, hopeless) or observation made by others (e.g., appears tearful). (Note: In children and adolescents, can be irritable mood.)

2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation).
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. (Note: In children, consider failure to make expected weight gain.)
4. Insomnia or hypersomnia nearly every day.
5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).
6. Fatigue or loss of energy nearly every day.
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

C. The episode is not attributable to the physiological effects of a substance or to another medical condition.

Note: Criteria A—C represent a major depressive episode.

Note: Responses to a significant loss (e.g., bereavement, financial ruin, losses from a natural disaster, a serious medical illness or disability) may include the feelings of intense sadness, rumination about the loss, insomnia, poor appetite, and weight loss noted in Criterion A, which may resemble a depressive episode. Although such symptoms may be understandable or considered appropriate to the loss, the presence of a major depressive episode in addition to the normal response to a significant loss should also be carefully considered. This decision inevitably requires the exercise of clinical judgment based on the individual's history and the cultural norms for the expression of distress in the context of loss.1

D. The occurrence of the major depressive episode is not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other specified and unspecified schizophrenia spectrum and other psychotic disorders.

E. There has never been a manic episode or a hypomanic episode.

Note: This exclusion does not apply if all of the manic-like or hypomanic-like episodes are substance-induced or are attributable to the physiological effects of another medical condition.

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1 In distinguishing grief from a major depressive episode (MDE), it is useful to consider that in grief the predominant affect is feelings of emptiness and loss, while in MDE it is persistent depressed mood and the inability to anticipate happiness or pleasure. The dysphoria in grief is likely to decrease in intensity over days to weeks and occurs in waves, the so-called pangs of grief.

These waves tend to be associated with thoughts or reminders of the deceased. The depressed mood of MDE is more persistent and not tied to specific thoughts or preoccupations. The pain of grief may be accompanied by positive emotions and humor that are uncharacteristic of the pervasive unhappiness and misery characteristic of MDE. The thought content associated with grief generally features a preoccupation with thoughts and memories of the deceased, rather than the self-critical or pessimistic ruminations seen in MDE. In grief, self-esteem is generally preserved, whereas in MDE feelings of worthlessness and self-loathing are common. If self derogatory ideation is present in grief, it typically involves perceived failings vis-ă-vis the deceased (e.g., not visiting frequently enough, not telling the deceased how much he or she was loved). If a bereaved individual thinks about death and dying, such thoughts are generally focused on the deceased and possibly about "joining" the deceased, whereas in MDE such thoughts are focused on ending one's own life because of feeling worthless, undeserving of life, or unable to cope with the pain of depression.

Coding and Recording Procedures

The diagnostic code for major depressive disorder is based on whether this is a single or recurrent episode, current severity, presence of psychotic features, and remission status. Current severity and psychotic features are only indicated if full criteria are currently met for a major depressive episode. Remission specifiers are only indicated if the full criteria are not currently met for a major depressive episode. Codes are as follows:

Severity/course specifier	Single episode	Recurrent episode*
Mild (p. 188)	296.21 (F32.O)	296.31 (F33.O)
Moderate (p. 188)	296.22 (F32.1)	296.32 (F33.1)
Severe (p. 188)	296.23 (F32.2)	296.33 (F33.2)
With psychotic features** (p. 186)	296.24 (F32.3)	296.34 (F33.3)
In partial remission (p. 188)	296.25 (F32.4)	296.35 (F33.41)
In full remission (p. 188)	296.26 (F32.5)	296.36 (F33.42)
Unspecified	296.20 (F32.9)	296.30 (F33.9)

*For an episode to be considered recurrent, there must be an interval of at least 2 consecutive months between separate episodes in which criteria are not met for a major depressive episode. The definitions of specifiers are found on the indicated pages.
**If psychotic features are present, code the "with psychotic features" specifier irrespective of episode severity.

In recording the name of a diagnosis, terms should be listed in the following order: major depressive disorder, single or recurrent episode, severity/psychotic/remission specifiers, followed by as many of the following specifiers without codes that apply to the current episode.

Specify:

With anxious distress (p. 184)
With mixed features (pp. 184—185)
With melancholic features (p. 185)
With atypical features (pp. 185—186)
With mood-congruent psychotic features (p. 186)
With mood-incongruent psychotic features (p. 186)
With catatonia (p. 186). Coding note: Use additional code 293.89 (F06.1). With peripartum onset (pp. 186—187)
With seasonal pattern (recurrent episode only) (pp. 187—188)

Diagnostic Features

The criterion symptoms for major depressive disorder must be present nearly every day to be considered present, with the exception of weight change and suicidal ideation. Depressed mood must be present for most of the day, in addition to being present nearly every day. Often insomnia or fatigue is the presenting

complaint, and failure to probe for accompanying depressive symptoms will result in underdiagnosis. Sadness may be denied at first but may be elicited through interview or inferred from facial expression and demeanor. With individuals who focus on a somatic complaint, clinicians should determine whether the distress from that complaint is associated with specific depressive symptoms. Fatigue and sleep disturbance are present in a high proportion of cases; psychomotor disturbances are much less common but are indicative of greater overall severity, as is the presence of delusional or near-delusional guilt.

The essential feature of a major depressive episode is a period of at least 2 weeks during which there is either depressed mood or the loss of interest or pleasure in nearly all activities (Criterion A). In children and adolescents, the mood may be irritable rather than sad. The individual must also experience at least four additional symptoms drawn from a list that includes changes in appetite or weight, sleep, and psychomotor activity; decreased energy; feelings of worthlessness or guilt; difficulty thinking, concentrating, or making decisions; or recurrent thoughts of death or suicidal ideation or suicide plans or attempts. To count toward a major depressive episode, a symptom must either be newly present or must have clearly worsened compared with the person's pre-episode status. The symptoms must persist for most of the day, nearly every day, for at least 2 consecutive weeks. The episode must be accompanied by clinically significant distress or impairment in social, occupational, or other important areas of functioning. For some individuals with milder episodes, functioning may appear to be normal but requires markedly increased effort.

The mood in a major depressive episode is often described by the person as depressed, sad, hopeless, discouraged, or "down in the dumps" (Criterion Al). In some cases, sadness may be denied at first but may subsequently be elicited by interview (e.g., by pointing out that the individual looks as if he or she is about to cry). In some individuals who complain of feeling "blah," having no feelings, or feeling anxious, the presence of a depressed mood can be inferred from the person's facial expression and demeanor. Some individuals emphasize somatic complaints (e.g., bodily aches and pains) rather than reporting feelings of sadness. Many individuals report or exhibit increased irritability (e.g., persistent anger, a tendency to respond to events with angry outbursts or blaming others, an exaggerated sense of frustration over minor matters). In children and adolescents, an irritable or cranky mood may develop rather than a sad or dejected mood. This presentation should be differentiated from a pattern of irritability when frustrated.

Loss of interest or pleasure is nearly always present, at least to some degree. Individuals may report feeling less interested in hobbies, "not caring anymore," or not feeling any enjoyment in activities that were previously considered pleasurable (Criterion A2). Family members often notice social withdrawal or neglect of pleasurable avocations (e.g., a formerly avid golfer no longer plays, a child who used to enjoy soccer finds excuses not to practice). In some individuals, there is a significant reduction from previous levels of sexual interest or desire.

Appetite change may involve either a reduction or increase. Some depressed individuals report that they have to force themselves to eat. Others may eat more and may crave specific foods (e.g., sweets or other carbohydrates). When appetite changes are severe (in either direction), there may be a significant loss or gain in weight, or, in children, a failure to make expected weight gains may be noted (Criterion A3).

Sleep disturbance may take the form of either difficulty sleeping or sleeping excessively (Criterion A4). When insomnia is present, it typically takes the form of middle insomnia (i.e., waking up during the night and then having difficulty returning to sleep) or terminal insomnia (i.e., waking too early and being unable to return to sleep). Initial insomnia (i.e., difficulty falling asleep) may also occur. Individuals who present with oversleeping (hypersomnia) may experience prolonged sleep episodes at night or increased daytime sleep. Sometimes the reason that the individual seeks treatment is for the disturbed sleep.

Psychomotor changes include agitation (e.g., the inability to sit still, pacing, handwringing; or pulling or rubbing of the skin, clothing, or other objects) or retardation (e.g., slowed speech, thinking, and body movements; increased pauses before answering; speech that is decreased in volume, inflection, amount, or variety of content, or muteness) (Criterion A5). The psychomotor agitation or retardation must be severe enough to be observable by others and not represent merely subjective feelings.

Decreased energy, tiredness, and fatigue are common (Criterion A6). A person may report sustained fatigue without physical exertion. Even the smallest tasks seem to require substantial effort. The efficiency with which tasks are accomplished may be reduced. For example, an individual may complain that washing and dressing in the morning are exhausting and take twice as long as usual.

The sense of worthlessness or guilt associated with a major depressive episode may include unrealistic negative evaluations of one's worth or guilty preoccupations or ruminations over minor past failings

(Criterion A7). Such individuals often misinterpret neutral or trivial day-to-day events as evidence of personal defects and have an exaggerated sense of responsibility for untoward events. The sense of worthlessness or guilt may be of delusional proportions (e.g., an individual who is convinced that he or she is personally responsible for world poverty). Blaming oneself for being sick and for failing to meet occupational or interpersonal responsibilities as a result of the depression is very common and, unless delusional, is not considered sufficient to meet this criterion.

Many individuals report impaired ability to think, concentrate, or make even minor decisions (Criterion A8). They may appear easily distracted or complain of memory difficulties. Those engaged in cognitively demanding pursuits are often unable to function. In children, a precipitous drop in grades may reflect poor concentration. In elderly individuals, memory difficulties may be the chief complaint and may be mistaken for early signs of a dementia ("pseudodementia"). When the major depressive episode is successfully treated, the memory problems often fully abate. However, in some individuals, particularly elderly persons, a major depressive episode may sometimes be the initial presentation of an irreversible dementia.

Thoughts of death, suicidal ideation, or suicide attempts (Criterion A9) are common. They may range from a passive wish not to awaken in the morning or a belief that others would be better off if the individual were dead, to transient but recurrent thoughts of committing suicide, to a specific suicide plan. More severely suicidal individuals may have put their affairs in order (e.g., updated wills, settled debts), acquired needed materials (e.g., a rope or a gun), and chosen a location and time to accomplish the suicide. Motivations for suicide may include a desire to give up in the face of perceived insurmountable obstacles, an intense wish to end what is perceived as an unending and excruciatingly painful emotional state, an inability to foresee any enjoyment in life, or the wish to not be a burden to others. The resolution of such thinking may be a more meaningful measure of diminished suicide risk than denial of further plans for suicide.

The evaluation of the symptoms of a major depressive episode is especially difficult when they occur in an individual who also has a general medical condition (e.g., cancer, stroke, myocardial infarction, diabetes, pregnancy). Some of the criterion signs and symptoms of a major depressive episode are identical to those of general medical conditions (e.g., weight loss with untreated diabetes; fatigue with cancer; hypersomnia early in pregnancy; insomnia later in pregnancy or the postpartum). Such symptoms count toward a major depressive diagnosis except when they are clearly and fully attributable to a general medical condition. Nonvegetative symptoms of dysphoria, anhedonia, guilt or worthlessness, impaired concentration or indecision, and suicidal thoughts should be assessed with particular care in such cases. Definitions of major depressive episodes that have been modified to include only these nonvegetative symptoms appear to identify nearly the same individuals as do the full criteria.

Associated Features Supporting Diagnosis

Major depressive disorder is associated with high mortality, much of which is accounted for by suicide; however, it is not the only cause. For example, depressed individuals admitted to nursing homes have a markedly increased likelihood of death in the first year. Individuals frequently present with tearfulness, irritability, brooding, obsessive rumination, anxiety, phobias, excessive worry over physical health, and complaints of pain (e.g., headaches; joint, abdominal, or other pains). In children, separation anxiety may occur.

Although an extensive literature exists describing neuroanatomical, neuroendocrinological, and neurophysiological correlates of major depressive disorder, no laboratory test has yielded results of sufficient sensitivity and specificity to be used as a diagnostic tool for this disorder. Until recently, hypothalamic-pituitary-adrenal axis hyperactivity had been the most extensively investigated abnormality associated with major depressive episodes, and it appears to be associated with melancholia, psychotic features, and risks for eventual suicide. Molecular studies have also implicated peripheral factors, including genetic variants in neurotrophic factors and pro-inflammatory cytokines. Additionally, functional magnetic resonance imaging studies provide evidence for functional abnormalities in specific neural systems supporting emotion processing, reward seeking, and emotion regulation in adults with major depression.

Prevalence

Twelve-month prevalence of major depressive disorder in the United States is approximately 7%, with marked differences by age group such that the prevalence 18- to 29-year-old individuals is threefold higher than the prevalence in individuals age 60 years or older. Females experience 1.5- to 3-fold higher rates than males beginning in early adolescence.

Development and Course

Major depressive disorder may first appear at any age, but the likelihood of onset increases markedly with puberty. In the United States, incidence appears to peak in the 20s; however, first onset in late life is not uncommon.

The course of major depressive disorder is quite variable, such that some individuals rarely, if ever, experience remission (a period of 2 or more months with no symptoms, or only one or two symptoms to no more than a mild degree), while others experience many years with few or no symptoms between discrete episodes. It is important to distinguish individuals who present for treatment during an exacerbation of a chronic depressive illness from those whose symptoms developed recently. Chronicity of depressive symptoms substantially increases the likelihood of underlying personality, anxiety, and substance use disorders and decreases the likelihood that treatment will be followed by full symptom resolution. It is therefore useful to ask individuals presenting with depressive symptoms to identify the last period of at least 2 months during which they were entirely free of depressive symptoms.

Recovery typically begins within 3 months of onset for two in five individuals with major depression and within 1 year for four in five individuals. Recency of onset is a strong determinant of the likelihood of near-term recovery, and many individuals who have been depressed only for several months can be expected to recover spontaneously. Features associated with lower recovery rates, other than current episode duration, include psychotic features, prominent anxiety, personality disorders, and symptom severity.

The risk of recurrence becomes progressively lower over time as the duration of remission increases. The risk is higher in individuals whose preceding episode was severe, in younger individuals, and in individuals who have already experienced multiple episodes. The persistence of even mild depressive symptoms during remission is a powerful predictor of recurrence.

Many bipolar illnesses begin with one or more depressive episodes, and a substantial proportion of individuals who initially appear to have major depressive disorder will prove, in time, to instead have a bipolar disorder. This is more likely in individuals with onset of the illness in adolescence, those with psychotic features, and those with a family history of bipolar illness. The presence of a "with mixed features" specifier also increases the risk for future manic or hypomanic diagnosis. Major depressive disorder, particularly with psychotic features, may also transition into schizophrenia, a change that is much more frequent than the reverse.

Despite consistent differences between genders in prevalence rates for depressive disorders, there appear to be no clear differences by gender in phenomenology, course, or treatment response. Similarly, there are no clear effects of current age on the course or treatment response of major depressive disorder. Some symptom differences exist, though, such that hypersomnia and hyperphagia are more likely in younger individuals, and melancholic symptoms, particularly psychomotor disturbances, are more common in older individuals. The likelihood of suicide attempts lessens in middle and late life, although the risk of completed suicide does not. Depressions with earlier ages at onset are more familial and more likely to involve personality disturbances. The course of major depressive disorder within individuals does not generally change with aging. Mean times to recovery appear to be stable over long periods, and the likelihood of being in an episode does not generally increase or decrease with time.

Risk and Prognostic Factors

Temperamental. Neuroticism (negative affectivity) is a well-established risk factor for the onset of major depressive disorder, and high levels appear to render individuals more likely to develop depressive episodes in response to stressful life events.

Environmental. Adverse childhood experiences, particularly when there are multiple experiences of diverse types, constitute a set of potent risk factors for major depressive disorder. Stressful life events are well recognized as precipitants of major depressive episodes, but the presence or absence of adverse life events near the onset of episodes does not appear to provide a useful guide to prognosis or treatment selection.

Genetic and physiological. First-degree family members of individuals with major depressive disorder have a risk for major depressive disorder two- to fourfold higher than that of the general population. Relative risks appear to be higher for early-onset and recurrent forms. Heritability is approximately 40%, and the personality trait neuroticism accounts for a substantial portion of this genetic liability.

Course modifiers. Essentially all major nonmood disorders increase the risk of an individual developing depression. Major depressive episodes that develop against the background of another disorder often follow a more refractory course. Substance use, anxiety, and borderline personality disorders are among the most common of these, and the presenting depressive symptoms may obscure and delay their recognition. However, sustained clinical improvement in depressive symptoms may depend on the appropriate treatment of underlying illnesses. Chronic or disabling medical conditions also increase risks for major depressive episodes. Such prevalent illnesses as diabetes, morbid obesity, and cardiovascular disease are often complicated by depressive episodes, and these episodes are more likely to become chronic than are depressive episodes in medically healthy individuals.

Culture-Related Diagnostic Issues

Surveys of major depressive disorder across diverse cultures have shown sevenfold differences in 12-month prevalence rates but much more consistency in female-to-male ratio, mean ages at onset, and the degree to which presence of the disorder raises the likelihood of comorbid substance abuse. While these findings suggest substantial cultural differences in the expression of major depressive disorder, they do not permit simple linkages between particular cultures and the likelihood of specific symptoms. Rather, clinicians should be aware that in most countries the majority of cases of depression go unrecognized in primary care settings and that in many cultures, somatic symptoms are very likely to constitute the presenting complaint. Among the Criterion A symptoms, insomnia and loss of energy are the most uniformly reported.

Gender-Related Diagnostic Issues

Although the mbst reproducible finding in the epidemiology of major depressive disorder has been a higher prevalence in females, there are no clear differences between genders in symptoms, course, treatment response, or functional consequences. In women, the risk for suicide attempts is higher, and the risk for suicide completion is lower. The disparity in suicide rate by gender is not as great among those with depressive disorders as it is in the population as a whole.

Suicide Risk

The possibility of suicidal behavior exists at all times during major depressive episodes. The most consistently described risk factor is a past history of suicide attempts or threats, but it should be remembered that most completed suicides are not preceded by unsuccessful attempts. Other features associated with an increased risk for completed suicide include male sex, being single or living alone, and having prominent feelings of hopelessness. The presence of borderline personality disorder markedly increases risk for future suicide attempts.

Functional Consequences of Major Depressive Disorder

Many of the functional consequences of major depressive disorder derive from individual symptoms. Impairment can be very mild, such that many of those who interact with the affected individual are unaware of depressive symptoms. Impairment may, however, range to complete incapacity such that the depressed individual is unable to attend to basic selfcare needs or is mute or catatonic. Among individuals seen in

general medical settings, those with major depressive disorder have more pain and physical illness and greater decreases in physical, social, and role functioning.

Differential Diagnosis

Manic episodes with irritable mood or mixed episodes. Major depressive episodes with prominent irritable mood may be difficult to distinguish from manic episodes with irritable mood or from mixed episodes. This distinction requires a careful clinical evaluation of the presence of manic symptoms.

Mood disorder due to another medical condition. A major depressive episode is the appropriate diagnosis if the mood disturbance is not judged, based on individual history, physical examination, and laboratory findings, to be the direct pathophysiological consequence of a specific medical condition (e.g., multiple sclerosis, stroke, hypothyroidism). Substance/medication-induced depressive or bipolar disorder. This disorder is distinguished from major depressive disorder by the fact that a substance (e.g., a drug of abuse, a medication, a toxin) appears to be etiologically related to the mood disturbance. For example, depressed mood that occurs only in the context of withdrawal from cocaine would be diagnosed as cocaine-induced depressive disorder.

Attention-deficit/hyperactivity disorder. Distractibility and low frustration tolerance can occur in both attention-deficit/ hyperactivity disorder and a major depressive episode; if the criteria are met for both, attention-deficit/ hyperactivity disorder may be diagnosed in addition to the mood disorder. However, the clinician must be cautious not to over diagnose a major depressive episode in children with attention-deficit/ hyperactivity disorder whose disturbance in mood is characterized by irritability rather than by sadness or loss of interest.

Adjustment disorder with depressed mood. A major depressive episode that occurs in response to a psychosocial stressor is distinguished from adjustment disorder with depressed mood by the fact that the full criteria for a major depressive episode are not met in adjustment disorder.

Sadness. Finally, periods of sadness are inherent aspects of the human experience. These periods should not be diagnosed as a major depressive episode unless criteria are met for severity (i.e., five out of nine symptoms), duration (i.e., most of the day, nearly every day for at least 2 weeks), and clinically significant distress or impairment. The diagnosis other specified depressive disorder may be appropriate for presentations of depressed mood with clinically significant impairment that do not meet criteria for duration or severity.

Comorbidity

Other disorders with which major depressive disorder frequently co-occurs are substance related disorders, panic disorder, obsessive-compulsive disorder, anorexia nervosa, bulimia nervosa, and borderline personality disorder.

Appendix D DSM V Anxiety disorder diagnostic criteria

Generalized Anxiety Disorder

Diagnostic Criteria 300.02 (F41.1)

A. Excessive anxiety and worry (apprehensive expectation), occurring more days than not for at least 6 months, about a number of events or activities (such as work or school performance).

B. The individual finds it difficult to control the worry.

C. The anxiety and worry are associated with three (or more) of the following six symptoms (with at least some symptoms having been present for more days than not for the past 6 months):

Note: Only one item is required in children.

1. Restlessness or feeling keyed up or on edge.
2. Being easily fatigued.
3. Difficulty concentrating or mind going blank.
4. Irritability.
5. Muscle tension.
6. Sleep disturbance (difficulty falling or staying asleep, or restless, unsatisfying sleep).

D. The anxiety, worry, or physical symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition (e.g., hyperthyroidism).

F. The disturbance is not better explained by another mental disorder (e.g., anxiety or worry about having panic attacks in panic disorder, negative evaluation in social anxiety disorder [social phobia], contamination or other obsessions in obsessive-compulsive disorder, separation from attachment figures in separation anxiety disorder, reminders of traumatic events in posttraumatic stress disorder, gaining weight in anorexia nervosa, physical complaints in somatic symptom disorder, perceived appearance flaws in body dysmorphic disorder, having a serious illness in illness anxiety disorder, or the content of delusional beliefs in schizophrenia or delusional disorder).

Diagnostic Features

The essential feature of generalized anxiety disorder is excessive anxiety and worry (apprehensive expectation) about a number of events or activities. The intensity, duration, or frequency of the anxiety and worry is out of proportion to the actual likelihood or impact of the anticipated event. The individual finds it difficult to control the worry and to keep worrisome thoughts from interfering with attention to tasks at hand. Adults with generalized anxiety disorder often worry about everyday, routine life circumstances, such as possible job responsibilities, health and finances, the health of family members, misfortune to their children, or minor matters (e.g., doing household chores or being late for appointments). Children with generalized anxiety disorder tend to worry excessively about their competence or the quality of their performance. During the course of the disorder, the focus of worry may shift from one concern to another.

Several features distinguish generalized anxiety disorder from nonpathological anxiety. First, the worries associated with generalized anxiety disorder are excessive and typically interfere significantly with psychosocial functioning, whereas the worries of everyday life are not excessive and are perceived as more manageable and may be put off when more pressing matters arise. Second, the worries associated with generalized anxiety disorder are

more pervasive, pronounced, and distressing; have longer duration; and frequently occur without precipitants. The greater the range of life circumstances about which a person worries (e.g., finånces, children's safety, job performance), the more likely his or her symptoms are to meet criteria for generalized anxiety disorder. Third, everyday worries are much less likely to be accompanied by physical symptoms (e.g., restlessness or feeling keyed up or on edge). Individuals with generalized anxiety disorder report subjective distress due to constant worry and related impairment in social, occupational, or other important areas of functioning.

The anxiety and worry are accompanied by at least three of the following additional symptoms: restlessness or feeling keyed up or on edge, being easily fatigued, difficulty concentrating or mind going blank, irritability, muscle tension, and disturbed sleep, although only one additional symptom is required in children.

Associated Features Supporting Diagnosis

Associated with muscle tension, there may be trembling, twitching, feeling shaky, and muscle aches or soreness. Many individuals with generalized anxiety disorder also experience somatic symptoms (e.g., sweating, nausea, diarrhea) and an exaggerated startle response. Symptoms of autonomic hyperarousal (e.g., accelerated heart rate, shortness of breath, dizziness) are less prominent in generalized anxiety disorder than in other anxiety disorders, such as panic disorder. Other conditions that may be associated with stress (e.g., irritable bowel syndrome, headaches) frequently accompany generalized anxiety disorder.

Prevalence

The 12-month prevalence of generalized anxiety disorder is 0.9% among adolescents and 2.9% among adults in the general community of the United States. The 12-month prevalence for the disorder in other countries ranges from 0.4% to 3.6%. The lifetime morbid risk is 9.0%. Females are twice as likely as males to experience generalized anxiety disorder. The prevalence of the diagnosis peaks in middle age and declines across the later years of life.

Individuals of European descent tend to experience generalized anxiety disorder more frequently than do individuals of non-European descent (i.e., Asian, African, Native American and Pacific Islander). Furthermore, individuals from developed countries are more likely than individuals from nondeveloped countries to report that they have experienced symptoms that meet criteria for generalized anxiety disorder in their lifetime.

Development and Course

Many individuals with generalized anxiety disorder report that they have felt anxious and nervous all of their lives. The median age at onset for generalized anxiety disorder is 30 years; however, age at onset is spread over a very broad range. The median age at onset is later than that for the other anxiety disorders. The symptoms of excessive worry and anxiety may occur early in life but are then manifested as an anxious temperament. Onset of the disorder rarely occurs prior to adolescence. The symptoms of generalized anxiety disorder tend to be chronic and wax and wane across the lifespan, fluctuating between syndromal and subsyndromal forms of the disorder. Rates of full remission are very low.

The clinical expression of generalized anxiety disorder is relatively consistent across the lifespan. The primary difference across age groups is in the content of the individual's worry. Children and adolescents tend to worry more about school and sporting performance, whereas older adults report greater concern about the well-being of family or their own physical heath. Thus, the content of an individual's worry tends to be age appropriate. Younger adults experience greater severity of symptoms than do older adults.

The earlier in life individuals have symptoms that meet criteria for generalized anxiety disorder, the more comorbidity they tend to have and the more impaired they are likely to be. The advent of chronic physical disease can be a potent issue for excessive worry in the elderly. In the frail elderly, worries about safety—and especially about falling—may limit activities. In those with early cognitive impairment, what appears to be excessive worry about, for example, the whereabouts of things is probably better regarded as realistic given the cognitive impairment.

In children and adolescents with generalized anxiety disorder, the anxieties and worries often concern the quality of their performance or competence at school or in sporting events, even when their performance is not being evaluated by others. There may be excessive concerns about punctuality. They may also worry about catastrophic events, such as earthquakes or nuclear war. Children with the disorder may be overly conforming, perfectionist, and unsure of themselves and tend to redo tasks because of excessive dissatisfaction with less-than-perfect performance. They are typically overzealous in seeking reassurance and approval and require excessive reassurance about their performance and other things they are worried about.

Generalized anxiety disorder may be overdiagnosed in children. When this diagnosis is being considered in children, a thorough evaluation for the presence of other childhood anxiety disorders and other mental disorders should be done to determine whether the worries may be better explained by one of these disorders. Separation anxiety disorder, social anxiety disorder (social phobia), and obsessive-compulsive disorder are often accompanied by worries that may mimic those described in generalized anxiety disorder. For example, a child with social anxiety disorder may be concerned about school performance because of fear of humiliation. Worries about illness may also be better explained by separation anxiety disorder or obsessive-compulsive disorder.

Risk and Prognostic Factors

Temperamental. Behavioral inhibition, negative affectivity (neuroticism), and harm avoidance have been associated with generalized anxiety disorder.

Environmental. Although childhood adversities and parental overprotection have been associated with generalized anxiety disorder, no environmental factors have been identified as specific to generalized anxiety disorder or necessary or sufficient for making the diagnosis.

Genetic and physiological. One-third of the risk of experiencing generalized anxiety disorder is genetic, and these genetic factors overlap with the risk of neuroticism and are shared with other anxiety and mood disorders, particularly major depressive disorder.

Culture-Related Diagnostic Issues

There is considerable cultural variation in the expression of generalized anxiety disorder. For example, in some cultures, somatic symptoms predominate in the expression of the disorder, whereas in other cultures cognitive symptoms tend to predominate. This difference may be more evident on initial presentation than subsequently, as more symptoms are reported over time. There is no information as to whether the propensity for excessive worrying is related to culture, although the topic being worried about can be culture specific. It is important to consider the social and cultural context when evaluating whether worries about certain situations are excessive.

Gender-Related Diagnostic Issues

In clinical settings, generalized anxiety disorder is diagnosed somewhat more frequently in females than in males (about 55%—60% of those presenting with the disorder are female). In epidemiological studies, approximately two-thirds are female. Females and males who experience generalized anxiety disorder appear to have similar symptoms but Generalized Anxiety Disorder 225

demonstrate different patterns of comorbidity consistent with gender differences in the prevalence of disorders. In females, comorbidity is largely confined to the anxiety disorders and unipolar depression, whereas in males, comorbidity is more likely to extend to the substance use disorders as well.

Functional Consequences of Generalized Anxiety Disorder

Excessive worrying impairs the individual's capacity to do things quickly and efficiently, whether at home or at work. The worrying takes time and energy; the associated symptoms of muscle tension and feeling keyed up or on edge, tiredness, difficulty concentrating, and disturbed sleep contribute to the impairment.

Importantly the excessive worrying may impair the ability of individuals with generalized anxiety disorder to encourage confidence in their children.

Generalized anxiety disorder is associated with significant disability and distress that is independent of comorbid disorders, and most non-institutionalized adults with the disorder are moderately to seriously disabled. Generalized anxiety disorder accounts for 110 million disability days per annum in the U.S. population.

Differential Diagnosis

Anxiety disorder due to another medical condition. The diagnosis of anxiety disorder associated with another medical condition should be assigned if the individual's anxiety and worry are judged, based on history, laboratory findings, or physical examination, to be a physiological effect of another specific medical condition (e.g., pheochromocytoma, hyperthyroidism).

Substance/medication-induced anxiety disorder. A substance/ medication-induced anxiety disorder is distinguished from generalized anxiety disorder by the fact that a substance or medication (e.g., a drug of abuse, exposure to a toxin) is judged to be etiologically related to the anxiety. For example, severe anxiety that occurs only in the context of heavy coffee consumption would be diagnosed as caffeine-induced anxiety disorder.

Social anxiety disorder. Individuals with social anxiety disorder often have anticipatory anxiety that is focused on upcoming social situations in which they must perform or be evaluated by others, whereas individuals with generalized anxiety disorder worry, whether or not they are being evaluated.

Obsessive-compulsive disorder. Several features distinguish the excessive worry of generalized anxiety disorder from the obsessional thoughts of obsessive-compulsive disorder. In generalized anxiety disorder the focus of the worry is about forthcoming problems, and it is the excessiveness of the worry about future events that is abnormal. In obsessive-compulsive disorder, the obsessions are inappropriate ideas that take the form of intrusive and unwanted thoughts, urges, or images.

Posttraumatic stress disorder and adjustment disorders. Anxiety is invariably present in posttraumatic stress disorder. Generalized anxiety disorder is not diagnosed if the anxiety and worry are better explained by symptoms of posttraumatic stress disorder. Anxiety may also be present in adjustment disorder, but this residual category should be used only when the criteria are not met for any other disorder (including generalized anxiety disorder). Moreover, in adjustment disorders, the anxiety occurs in response to an identifiable stressor within 3 months of the onset of the stressor and does not persist for more than 6 months after the termination of the stressor or its consequences.

Depressive, bipolar, and psychotic disorders. Generalized anxiety/ worry is a common associated feature of depressive, bipolar, and psychotic disorders and should not be diagnosed separately if the excessive worry has occurred only during the course of these conditions.

Comorbidity

Individuals whose presentation meets criteria for generalized anxiety disorder are likely to have met, or currently meet, criteria for other anxiety and unipolar depressive disorders. The neuroticism or emotional liability that underpins this pattern of comorbidity is associated with temperamental antecedents and genetic and environmental risk factors shared between these disorders, although independent pathways are also possible. Comorbidity with substance use, conduct, psychotic, neurodevelopmental, and neurocognitive disorders is less common.

MONTGOMERY-ASBERG DEPRESSION RATING SCALE (MADRS)

OVERALL SEVERITY

The rating should be based on a clinical interview moving from broadly phrased questions about symptoms to more detailed ones which allow a precise rating of severity. The rater must decide whether the rating lies on the defined scale steps (0, 2, 4, 6) or between them (1 , 3, 5).

It is important to remember that it is only on rare occasions when a depressed patient is encountered who cannot be rated on the items on the scale. If definite answers cannot be elicited from the patient all relevant clues as well as information from other sources should be used as a basis for the rating in line with customary clinical practice. The scale may be used for any time interval between ratings, be it weekly or otherwise but this must be recorded.

Specify one of the reasons listed below by putting appropriate number in adjacent box.

1 . APPARENT SADNESS

Representing despondency, gloom, and despair (more than just ordinary transient low spirits) reflected in speech, facial expression, and posture. Rate by depth and inability to brighten up.

0 - No sadness

1

2 - Looks dispirited but does brighten up without difficulty 3

4 - Appears sad and unhappy most of the time

5

6 - Looks miserable all the time. Extremely despondent

2. REPORTED SADNESS

Representing reports of depressed mood, regardless of whether it is reflected in appearance or not. Includes low spirits, despondency, or the feeling of being beyond help and without hope. Rate according to intensity, duration, and the extent to which the mood is reported to be influenced by events.

0 - Occasional sadness in keeping with the circumstances

1

2 - Sad or low but brightens up without difficulty 3

4 - Pervasive feelings of sadness or gloominess. The mood is still influenced by external circumstances

5

6 - Continuous or unvarying sadness, misery, or despondency

3. INNER TENSION

Representing feelings of ill-defined discomfort, edginess, inner turmoil, mental tension mounting to either panic, dread, or anguish.

Rate according to intensity, frequency, duration, and the extent of reassurance called for. 0 -

Placid. Only fleeting inner tension

1

2 - Occasional feelings of edginess and ill-defined discomfort 3

4 - Continuous feelings of inner tension or intermittent panic which the patient can only master with some difficulty

5

6 - Unrelenting dread or anguish. Overwhelming panic

4. REDUCED SLEEP

Representing the experience of reduced duration or depth of sleep compared to the patient's own normal pattern when well.

0 - Sleeps as usual

1

2 - Slight difficulty dropping off to sleep or slightly reduced, light or fitful sleep

3

4 - Sleep reduced or broken by at least 2 hours

5

6 - Less than 2 or 3 hours sleep

5. REDUCED APPETITE

Representing the feeling of a loss of appetite compared with when well. Rate by loss of desire for food or the need to force oneself to eat.

0 - Normal or increased appetite

1

2 - Slightly reduced appetite

3

4 - No appetite. Food is tasteless

5

6 - Needs persuasion to eat at all

6. CONCENTRATION DIFFICULTIES

Representing difficulties in collecting one's thoughts mounting to incapacitating lack of concentration.

Rate according to intensity, frequency, and degree of incapacity produced.

0 - No difficulties in concentrating

1

2 - Occasional difficulties in collecting one's thoughts 3

4 - Difficulties in concentrating and sustaining thought which reduces ability to read or hold a conversation

5

6 - Unable to read or converse without great difficulty

7. LASSITUDE

Representing a difficulty getting started or slowness initiating and performing everyday activities.

0 - Hardly any difficulty in getting started. No sluggishness

1

2 - Difficulties in starting activities

3

4 - Difficulties in starting simple routine activities which are carried out with effort

5

6 - Complete lassitude. Unable to do anything without help

8. INABILITY TO FEEL

Representing the subjective experience of reduced interest in the surroundings, or activities that normally give pleasure. The ability to react with adequate emotion to circumstances or people is reduced.

0 - Normal interest in surroundings and in other people

1

2 - Reduced ability to enjoy usual interests 3

4 - Loss of interest in the surroundings. Loss of feelings for friends and acquaintances

5

6 - The experience of being emotionally paralyzed, inability to feel anger, grief, or pleasure and a complete or even painful failure to feel for close relatives and friends

9. PESSIMISTIC THOUGHTS

Representing thoughts of guilt, inferiority, self-reproach, sinfulness, remorse, and ruin.

0 - No pessimistic thoughts

1

2 - Fluctuating ideas of failure, self-reproach, or self-depreciation 3

4 - Persistent self-accusations or definite but still rational ideas of guilt or sin. Increasingly pessimistic about the future

5

6 - Delusions of ruin, remorse, or unredeemable sin. Self-accusations which are absurd and unshakable

10. SUICIDAL THOUGHTS

Representing the feeling that life is not worth living, that a natural death would be welcome, suicidal thoughts, and preparations for suicide. Suicide attempts should not in themselves influence the rating.

0 - Enjoys life or takes it as it comes

1

2 - Weary of life. Only fleeting suicidal thoughts 3

4 - Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intention

5

6 - Explicit plans for suicide when there is an opportunity. Active preparations for suicide

Scoring and Interpretation:

Higher scores indicate increasing depressive symptoms. Ratings can be added to form an overall score (range 0 to 50).

0 to 6 – symptom absent

7 to 19 – mild depression

20 to 34 – moderate

35 to 60 – severe depression

Information provided by Western University

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:

Total score: Depression (D) _____ Anxiety (A) _____

0-7 = Normal 8-10 = Borderline abnormal (borderline case) 11-21 = Abnormal (case)

Appendix G 24-hour Diet Recall Form

24-hour Diet Recall Form

Participants Identification Code:

Initials of Participant:

Date:

Time of Day	Food or Beverage Consumed	Quantity/amount consumed	Method of preparation, Brand Name	Where was it consumed

Appendix H Mediterranean Diet Adherence Screener (MEDAS)

Olive oil as the principal source of fat for cooking	**+1**
Four or more tablespoons—1 tablespoon 13.5 g— of olive oil/day (including that used in frying, salads, meals eaten away from home)	+1
Two or more serving of vegetables/day	+1
Three or more pieces of fruit/day	+1
<1 Serving of red meat or sausages/day	+1
<1 Serving of animal fat/day	+1
<1 Cup (1 cup = 100 ml) of sugar-sweetened beverages/day	+1
Three or more servings of fish/week	+1
Three or more serving of nuts/week(30g/serving)	+1
<2 Commercial pastries/week	+1
Two or more servings/week of a dish with a traditional sauce of tomatoes, garlic, onion, or leeks sautéed in olive oi.	+1
Preferring white meat over red meat	+1

A score less than 8 is considered poor dietary compliance.

A score greater than 8 is considered to be satisfactory compliance.

Appendix I Modified Mediterranean Diet Recipes

Couscous with Sun-Dried Tomato and Feta

This easy couscous recipe works well served warm or at room temperature.

Prep Time 5 minutes
Cook Time 5 minutes
Total Time 10 minutes

Servings 4

Ingredients

- 1/3 cup shelled pine nuts
- 1 tablespoon olive oil
- 1/2 teaspoon kosher salt
- 1 1/2 cup couscous
- 1/3 cup sun-dried tomatoes in oil, drained and diced
- 1/3 cup crumbled feta cheese
- 1/4 cup chopped green onion

Instructions

1. In a dry, non-stick fry pan over medium-high heat, toast the pine nuts, tossing often, until golden brown, about 3-4 minutes. Be sure to watch them closely as they can burn quickly once they get hot. Set aside.
2. In a medium saucepan, bring 1 1/4 cup water to a boil. Stir in the couscous, olive oil and kosher salt, cover, and remove from the heat. Let stand for 5 minutes.
3. Fluff the couscous with a fork and stir in the sun-dried tomatoes, feta cheese, chopped green onion, and pine nuts. This dish can be served warm or at room temperature.

Mediterranean Quinoa Salad

This healthy vegetarian quinoa salad makes for a simple lunch or dinner, thanks to staples like roasted red bell peppers, kalamata olives, and feta from your fridge and pantry.

Prep Time 15 minutes

Total Time 15 minutes

Servings 4

Ingredients

- 1 1/2 cups dry quinoa
- 1/2 teaspoon kosher salt
- 1/2 cup extra virgin olive oil
- 1 tablespoon balsamic vinegar
- 2 garlic cloves pressed
- 1/2 teaspoon dry basil minced
- 1/2 teaspoon dried thyme crushed between your fingers
- kosher salt and freshly ground black pepper
- 3 cups arugula
- 1 15 ounce can garbanzo beans drained
- 1 package DeLallo Zesty Salad Savors

Instructions

1. Cook the quinoa according to package directions with 1/2 teaspoon salt added to the water. Cool completely.
2. Mix together the olive oil, balsamic vinegar, pressed garlic, basil, and thyme. Whisk until well combined. Season with kosher salt and freshly ground black pepper and set aside.
3. To a large serving bowl, add the quinoa, arugula, garbanzo beans, and contents of the Salad Savors package (red bell pepper, kalamata olives and feta cheese).
4. Drizzle with the dressing and garnish with basil. Season to taste. Serve at room temperature.

Recipe Notes

*Instead of a packet of Zesty Salad Savors, you can use the following instead:

- Small jar roasted red bell peppers (drained and chopped)
- 1/4 cup kalamata olives (roughly chopped)
- 1/4 cup crumbled feta cheese

THE BEST Greek Chicken Marinade

This garlic, lemon, and oregano Greek marinade can be used for chicken that's grilled, baked or pan-fried and is great any which way you choose.

Prep Time 5 minutes

Cook Time 15 minutes

Marinate the chicken 30 minutes

Total Time 50 minutes

Servings 4

Ingredients

- 1-pound boneless skinless chicken breasts (about 2 large breasts)
- ⅓ cup plain Greek yogurt
- ¼ cup olive oil
- 4 lemons
- 4-5 cloves garlic pressed or minced
- 2 tablespoons dried oregano
- 1 teaspoon kosher salt
- ½ teaspoon freshly ground black pepper

Instructions

1. Place the chicken pieces in a freezer bag or a bowl and set aside.
2. Add the Greek yogurt and olive oil to a medium size bowl. Zest one of the lemons and add to the bowl. Juice that lemon into the bowl with the zest. Slice the other three lemons and set aside. Add the minced garlic, oregano, kosher salt and black pepper to the lemon juice and zest and stir. Pour half of the marinade into the freezer bag or the bowl with the chicken pieces and reserve the other half of the marinade for basting. Marinate the chicken for 30 minutes or up to 3 hours in the refrigerator.
3. When ready to grill, prepare the grill by lightly oiling the grate with vegetable oil or cooking spray and set to medium high heat.
4. Grill the chicken, basting with the reserved marinade and turning often so each side browns and has light grill marks, until cooked through, about 15-20 minutes or until the chicken juices run clear. During the last 5 minutes of cooking add the 3 sliced lemons to the grill, turning once or twice. Allow the chicken to rest for 5 minutes before slicing and serve with the grilled lemons. Refrigerate leftovers for up to 3 days.

Mediterranean Chicken Quinoa Bowl with Broccoli and Tomato

Chicken quinoa bowls have staked their healthy claim on quick and easy meal-prep dinners that are flexible enough to mix and match ingredients for take-to-work lunches that never get boring, too.

Prep Time 10 minutes

Cook Time 30 minutes

Total Time 40 minutes

Servings 3

Ingredients

For the Chicken:

- 1 6- ounce skinless, boneless chicken breast
- 1/4 cup + 2 tablespoons olive oil
- 1 lemon juiced and zested
- 2 cloves garlic pressed or minced
- 2 teaspoons dried oregano
- 1/2 teaspoon kosher salt
- 1/4 teaspoon freshly ground black pepper
- 1 cup Easy Roasted Broccoli and Feta
- 1/2 cup Easy Roasted Tomatoes

For the Quinoa:

- 1 cup dried quinoa
- 1 teaspoon kosher salt
- Crumbled feta cheese

Instructions

1. Slice the chicken breast into 1-inch chunks and add to a gallon freezer bag. In a small bowl, whisk the olive oil, lemon juice and zest, garlic, oregano and salt and pepper then add to the bag, seal, and marinade for at least 30 minutes up to overnight.
2. Heat the remaining 2 tablespoons olive oil in a non-stick skillet over medium high heat. Add the chicken to the skillet and cook until browned on all sides and cooked through, about 10-12 minutes.
3. Reduce the heat to medium and add the broccoli and tomatoes to the pan with more olive oil if needed, and warm through.
4. Meanwhile, cook the quinoa. Rinse it in a fine mesh strainer under cold water first. Bring a saucepan of water to a boil over high heat, then add 1 teaspoon of kosher salt and the quinoa. Boil it like pasta, until al dente, stirring occasionally, about 8 to

10 minutes. Drain, fluff with a fork, and return the quinoa to the pot, cover with a kitchen towel, then a lid and let sit for 5-10 minutes.

5. To assemble the bowls, divide the quinoa between the bowls and top each with half of the chicken and vegetable mixture. Season with more kosher salt and freshly ground black pepper to taste and drizzle with more olive oil if you'd like. Sprinkle with feta cheese crumbles and serve.

Recipe Notes

The roasted broccoli and roasted tomatoes can be made ahead and stored in the refrigerator for up to 3 days before adding to the cooked chicken to warm before composing the quinoa bowls.

Tables

Table 3

Research Questions and Variables

Research Question	**Independent**	**Dependent**	**Confounding**
To what extent does altering the microbiome in individuals diagnosed with major depressive disorder or anxiety through, improved diet quality, impact symptom severity on anxiety and depression assessment scores?	Diet quality	Symptom severity of anxiety and depression assessment scores Microbiome bacterial populations	medications
Does improving diet quality in individuals diagnosed with MDD or anxiety, by following a modified Mediterranean diet, alter microbiome bacterial populations?	Diet quality	Microbiome bacterial populations	medications
Does improving diet quality in individuals diagnosed with MDD or anxiety, through a modified Mediterranean diet, impact symptom severity on anxiety and depression assessment scores?	Diet quality	Symptom severity of anxiety and depression assessment scores	medications

Table 4

Inferential Statistics

Research Question	Independent Variable?	Potential Responses	Level of Measurement	Dependent Variable?	Potential Responses	Level of Measurement	Test of Significance
Main	Diet quality	Compliant Non-compliant	categorical	Symptom Severity of MDD and GAD assessments Altered microbiome populations	0-60 Or 0-21 Continuous	numerical	MANOVA
Sub 1	Diet Quality	0-13	numerical	Microbiome populations	continuous	numerical	paired T test
Sub 2	Diet Quality	0-13	numerical	Symptom Severity of MDD and GAD assessments Week 6	0-60 or 0-21	numerical	Paired T test

Table 5

Baseline Characteristics of dietary intervention or habitual diet groups

		Total (n=127)	DI (n=64)	HD (n=63)	P value
Demographic					
Gender % female	%(n)	55(70)	58(38)	52 (32)	0.72
Age	M(SD)	30.4 (6.9)	30.1 (6.8)	30.6 (7.1)	0.73
Health Measures					
BMI	M(SD)	26.8 (5.1)	26.9 (5.3)	26.6 (4.9)	0.74
Diet Quality					
MEDAS (0-12)	M(SD)	5.3 (3.1)	5.2 (2.1)	5.3 (2.1)	0.80
Psychological Measures					
MADRS (0-60)	M(SD)	26.4 (2.9)	26.3 (2.9)	26.5 (2.9)	0.77
HADS-D (0-21)	M(SD)	8.6 (1.3)	8.6 (1.2)	8.7 (1.3)	0.60
HADS-A (0-21)	M(SD)	11.3 (1.6)	11.3 (1.6)	11.4 (1.6)	0.77

Note: *BMI* body mass index, *MEDAS* Mediterranean Diet Adherence Screener, *MADRS* Montgomery-Asberg Depression Rating Scale, *HADS* Hospital Anxiety and Depression Scale

*p<0.5 indicates statistical significance

Table 6

Change in Psychological symptom and MEDAS scores

Test Score	Dietary Intervention			Habitual Diet			Change between groups at 6 weeks p-value
	Baseline M(SD)	6 weeks M(SD)	p-value	Baseline M(SD)	6 weeks M(SD)	p-value	
Symptom Severity							
MADRS (0-60)	26.3(2.9)	17.4(3.7)	<0.001	26.5(2.9)	19.5(2.3)	<0.001	<0.001
HADS-D (0-21)	8.6(1.2)	7.1(1.1)	<0.001	8.7(1.3)	8.3(1.4)	<0.001	0.035
HADS-A (0-21)	11.3(1.6)	8.7(1.3)	<0.001	11.4(1.6)	10.3(1.6)	<0.001	<0.001
Diet							

MEDAS Score	5.2(2.1)	7.9(1.4)	<0.001	5.3(2.1)	7.0 (1.4)	<0.001	<0.001

Note: p-value < 0.05 is considered significant.

Table 7

Microbiome Population Percentages at baseline, week three, and week six

	Dietary Intervention				Habitual Diet				Change between Groups at Week 6 P Value
Bacteria	Baseline	Week 3	Week 6	Change from Baseline to Week 6 P Value	Baseline	Week 3	Week 6	Change from Baseline to Week 6 P Value	
Bifidobacterium % pop (SD)	9.8(1.3)	10.9(1.2)	11.9(1.1)	<0.001	9.8(1.2)	11.2(1.1)	10.9(1.1)	<0.001	<0.001
Proteobacteria % pop (SD)	0.9(0.5)	1.1(0.6)	0.8(0.5)	0.32	0.9(0.6)	0.9(0.6)	1.0(0.6)	0.50	0.68
Streptococcus % pop (SD)	1.1(0.6)	1.0(0.6)	0.7(0.5)	0.003	1.1(0.6)	0.9(0.6)	1.0(0.6)	0.53	0.01
Lachnospiraceae % pop (SD)	0.9(0.6)	1.9(0.6)	2.4(0.6)	<0.001	1.0(0.6)	1.0(0.6)	1.0(0.6)	0.16	<0.001
Ruminococcus % pop (SD)	1.0(0.6)	1.9(0.6)	2.4(0.6)	<0.001	1.0(0.6)	1.0(0.5)	1.0(0.5)	0.002	<0.001

Note: p-value < 0.05 is considered significant.

Table 8

MANOVA Descriptive Statistics

	Dietary Compliance	Mean	Std. Deviation	N
MADRS Scores	compliant	18.75	3.605	68
	non-compliant	18.10	2.796	59
	Total	18.45	3.258	127
HADS-A Scores	compliant	9.01	1.501	68
	non-compliant	9.56	1.600	59
	Total	9.27	1.566	127
HADS-D Scores	compliant	7.24	1.283	68
	non-compliant	7.42	1.367	59
	Total	7.32	1.321	127
Bifidobacterium Population %	compliant	11.49	1.174	68
	non-compliant	11.24	1.283	59
	Total	11.38	1.227	127
Proteobacteria Population %	compliant	.82	.472	68
	non-compliant	.84	.468	59
	Total	.83	.469	127
Streptococcus Population %	compliant	.84	.507	68
	non-compliant	.90	.590	59
	Total	.87	.546	127
Lachnospiraceae Population %	compliant	1.75	.972	68
	non-compliant	1.53	.960	59
	Total	1.65	.969	127
Ruminococcus Population %	compliant	1.62	1.084	68
	non-compliant	1.61	.960	59
	Total	1.61	1.024	127

Note: p-value < 0.05 is considered significant.

Table 9

Test of Between-Subject Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	MADRS	13.278[a]	1	13.28	1.253	.265	.010
	HADS-A	9.37[b]	1	9.37	3.910	.050	.030
	HADS-D	1.12[c]	1	1.12	.641	.425	.005
	Bifidobacterium	2.00[d]	1	2.00	1.333	.251	.011
	Proteobacteria	.02[e]	1	.02	.098	.755	.001
	Streptococcus	.14[f]	1	.14	.465	.496	.004
	Lachnospiraceae	1.55[g]	1	1.55	1.656	.201	.013
	Ruminococcus	.003[h]	1	.003	.003	.960	.000
Intercept	MADRS	42901.47	1	42901.47	4049.94	<.001	.970
	HADS-A	10898.57	1	10898.58	4548.23	<.001	.973
	HADS-D	6788.40	1	6788.40	3881	<.001	.969
	Bifidobacterium	16330.67	1	16330.67	10871.9	<.001	.989
	Proteobacteria	86.90	1	86.90	392.76	<.001	.759
	Streptococcus	95.31	1	95.31	318.52	<.001	.718
	Lachnospiraceae	339.62	1	339.62	363.80	<.001	.744
	Ruminococcus	328.79	1	328.79	310.83	<.001	.713
Compliance	MADRS	13.28	1	13.28	1.25	.265	.010
	HADS-A	9.370	1	9.37	3.91	.050	.030
	HADS-D	1.12	1	1.12	.64	.425	.005
	Bifidobacterium	2.00	1	2.00	1.33	.251	.011
	Proteobacteria	.022	1	.02	.098	.755	.001
	Streptococcus	.139	1	.14	.465	.496	.004
	Lachnospiraceae	1.55	1	1.55	1.656	.201	.013
	Ruminococcus	.003	1	.003	.003	.960	.000

a. R Squared = .010 (Adjusted R Squared = .002)
b. R Squared = .030 (Adjusted R Squared = .023)
c. R Squared = .005 (Adjusted R Squared = -.003)
d. R Squared = .011 (Adjusted R Squared = .003)
e. R Squared = .001 (Adjusted R Squared = -.007)
f. R Squared = .004 (Adjusted R Squared = -.004)
g. R Squared = .013 (Adjusted R Squared = .005)
h. R Squared = .000 (Adjusted R Squared = -.008)

Note: p-value < 0.05 is considered significant.

Figures

Figure 1

Flowchart illustration for the study participants