Mood Disturbance and the Gut Microbiome

by

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Abstract

Microbiota residing in the gastrointestinal tract play a crucial role in physical and mental health of the human host. The gut microbiome affects immunity and inflammation, absorption and digestion of nutrients, production of neuromodulators, and central nervous system function. Microbial diversity is theorized to benefit human health through a range of genes expressed by over 1500 species of microbes. Exploration into the relationship between the gut microbiome and human health has considerably grown in recent years, including a focus on mood and mood-related disorders. Gut microbiota influence communication and signaling in the central nervous system along the gut-brain axis, impacting the response to stress, mood, and quality of life. Most supporting data in this area comes from preclinical studies and a limited number of unique human populations. Herein, we explored the relationship between the gut microbiome and quality of life and mood disturbance in breast cancer survivors, free-living adults, and young adults enrolled in a randomized controlled trial.

The first study found differences in fecal microbiome composition between obese and non-obese breast cancer survivors. Several genera previously identified as beneficial were associated with quality-of-life components. In the second study, greater microbial diversity was associated with lower mood disturbance in free-living adults. Quality of diet was also associated with mood states in this study. The third study investigated changes in microbial diversity, dietary components, and body composition, and the relationships between these variables, following a 10-week resistance training regimen. Several microbial taxa associated with mood states in the intervention groups, and overall, microbial diversity increased following the training regimen.

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Abbreviations

ASA-24	Automated Self-Administered 24-hour recall
ASVs	Amplicon Sequence Variants
BBB	Blood Brain Barrier
BCS	Breast Cancer Survivors
BIA	Body Impedance Analysis
BMI	Body Mass Index
BP	Blood Pressure
CNS	Central Nervous System
CRE	Cardiorespiratory
CTL	Control
DEXA	Dual energy x-ray absorptiometry
DIM	Diet, Inflammation, Microbes
DQ	Diet Quality
FDR	False Discovery Rate
FFM	Fat-free Mass
FMT	Fecal Microbiota Transplants
GABA	Gamma-aminobutyric acid
GBA	Gut-brain axis
GI	Gastrointestinal
HEI	Healthy Eating Index
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
LPS	Lipopolysaccharide
M3G	Meat and Three Greens
MAMPs	Microbe-associated molecular patterns
MCS	Mental Component Score
MD	Mood Disturbance
MDD	Major Depressive Disorder
NCI	National Cancer Institute
NDSR	Nutrition Data System for Research
PA	Physical Activity
PAMPs	Pathogen-associated molecular patterns
POMS	Profile of Mood States
PP	Peanut Protein
PPS	Peanut Protein Supplementation
PRR	Pattern Recognition Receptors
QOL	Quality of Life
RD	Registered Dietitian
RT	Resistance Training
SCFAs	Short Chain Fatty Acids

SD	Standard Deviation
SERA	Self-Esteem-Related-Affect
SF-36	Short-Form Health Survey
TJ	Tight Junctions
US	United States

Chapter 1: Introduction

The gut microbiome is defined as the collective genome of the microorganisms residing in the gastrointestinal (GI) tract. Gut microbial cells match human body cells in quantity and encode 150 times more genes than the human genome [1-3]. Composition of these microbes greatly impacts human health [4], including immune system priming and maturation [5], nutrient digestion and absorption [6], gut and blood-brain barrier integrity [7, 8], and neurotransmitter production [9]. More microbial diversity is associated with better physical and mental health [10-12]. Gut microbiota directly interact with the intestinal physiology and affect immune and inflammatory response and production of cytokines [13]. Commensal bacteria have a symbiotic relationship with the human host [5], and can protect the gut from imbalanced diversity, called dysbiosis [14]. Dysregulation of the immune system due to dysbiosis can contribute to tumor development [15], rheumatoid arthritis [16], multiple sclerosis [17], and other disorders [4]. Several studies have observed reduced microbial diversity and altered microbiome composition in individuals with obesity compared to lean individuals [18-20], as well as altered microbiome composition in patients with type 2 diabetes compared to healthy controls [21]. Microbiome diversity is associated with cognitive outcomes and brain connectivity as early as 2 years of age [22]. Moreover, microbial diversity is decreased in patients with Major Depressive Disorder (MDD) in comparison to healthy controls [11, 23].

The bidirectional communication of the gut microbiome and the central nervous system (CNS) occurs along the gut-brain-axis (GBA). Numerous preclinical studies demonstrate the effects of gut microbiota on behavior and neurochemical production by utilizing germ-free models [24, 25], antibiotic-depleted mice [26, 27], and fecal microbiome transplants (FMTs) [23, 28, 29].

In humans, anxiety and depression symptoms are comorbid with Inflammatory Bowel Diseases (IBD) [30] and microbial similarities are observed between patients with depression and Irritable Bowel Syndrome (IBS) [31]. There is an established relationship between functional gut disorders and anxiety and depression and their related symptoms [32-34]. Seemingly, altering the gut microbiota may yield beneficial mental health outcomes. Composition of the gut microbiome is influenced by a range of both fixed and modifiable factors including host genetics [35], mode of delivery [36], environmental elements [37, 38], use of antibiotics [39], dietary factors [40, 41], and physical activity (PA) [42, 43].

Diet and exercise are modifiable factors that can alter composition of the gut microbiome and are common lifestyle modifications suggested by healthcare providers treating mood disorders, which include obsessive-compulsive disorder, MDD, anxiety disorder, and bipolar disorder [44]. Omega-3 polyunsaturated fatty acids are suggested to be protective against mood disorders [45], and can also influence the composition of the gut microbiome [46, 47]. Likewise, fruit and vegetable consumption may reduce psychological distress [48, 49] while improving the gut microbial composition [50, 51]. Additionally, PA can alter the gut microbiome and induce changes in cognition and anxiety [52]. Aerobic training is thought to have the greatest impact on microbiota, although research in human populations is limited [53, 54]. Engaging in PA is associated with improved anxiety and depression symptoms [55-57].

Depression and anxiety symptoms are aspects of mood disturbance, along with feelings of fatigue, anger, and sadness, that contribute to the pathophysiology of mood disorders [58]. Similarly, quality of life (QOL) includes social, mental, and physical health and well-being [59]. Distinctive in mood disorders, persistent mood disturbance leads to emotional inflexibility and diminished QOL [60]. Mood disorders come with higher indirect and direct healthcare costs and

are some of the leading causes of disability across the globe [61]. Mortality risk for comorbid conditions is higher in individuals with a mood disorder diagnosis [62]. Outside of mood disorders, experiencing stress and mood disturbance is a perpetual aspect of humanity and the behavioral and physiological response to these daily stressors is of import.

The same modifiable lifestyle habits that alter the composition of the gut microbiome (i.e., diet and PA) can also improve mood disturbance and stress response [63]. Dietary interventions have successfully reduced depression symptoms [64-66] and anxiety [67], and engaging in PA improves mood comparable to anti-depressant medications [68, 69]. While multiple mechanisms are involved in the mood responses to PA specific gut microbial changes have been implicated [70, 71]. Investigating the relationship between diet, PA, mood, and the gut microbiome provides insight into feasible lifestyle modifications for humans to strengthen their response to stress, gain emotional flexibility, and diminish risk for persistent mood disturbance. Herein, the studies explore the connection between mood, diet, PA, and gut microbiota in various populations. We hypothesized greater microbial diversity would be associated with lower mood disturbance. Diet and physical activity will influence the relationship between the fecal microbiome and mood disturbance dimensions.

Chapter 2: Review of Literature

2.1 Introduction

This review of literature provides an overview of the gut microbiome, mood and related disorders, and their connection via the gut-brain axis (GBA). First, the gut microbiome is defined, and its impact on human health is discussed. Next, mood disturbance and disorders are examined, as well as the challenges and issues surrounding prevalence and treatment avenues. Lastly, the GBA is defined and discussed in the context of mood disturbance and disorders and the therapeutic potential of manipulating the gut microbiome.

2.2 Gut Microbiome

2.2.1 Definitions

The gut microbiome is a complex collection of microorganisms residing within the GI tract. Compared to skin and oral microbiomes, the GI tract is home to the greatest number of species [72] and can contain bacteria, viruses, archaea, and some eukaryotes [1]. Many factors contribute to the composition of the microbiome, including genetics of host [73], mode of delivery [36], environmental elements [74], antibiotic use [39], dietary habits and components [75], pro- or prebiotic consumption [76, 77], and exercise [42]. The first major exposure to microbiota occurs during labor and birth, and it is thought that microbial diversity reaches levels of an adult by the second or third year of life [78, 79]. Prior to advances in gene sequencing, approximately 60-80% of gut microbes could not be cultured and therefore were difficult to identify [80]. High throughput DNA sequencing has allowed thorough investigation of the symbiotic relationship between these gut microbes and human host health. Interest in the gut microbiome has grown expansively in the past two decades [81], particularly after large-scale metagenomic projects, such as the Human

Microbiome Project [3] and the Metagenomics of the Human Intestinal Tract (MetaHIT) [82]. From these studies, scientists determined that the microbiome encodes approximately 150 times more genes than the human genome [3], is comprised of more than 1,500 species and 50 phyla [1], and is composed predominantly of strict anaerobes and some facultative anaerobes due to the lack of oxygen found in the GI tract [83].

Besides matching human cells 1 to 1, gut microbiota also play a crucial role in health through multiple pathways [2, 84]. Certain taxa have a symbiotic relationship with the human host, and these are considered commensal or beneficial microbes [5]. An interspecies balance of commensal microbes is considered eubiosis, while a disruption of this is called dysbiosis [85]. Dysbiosis can be characterized by reduced abundance of key taxa, reduced alpha diversity, increased abundance of pathogenic bacteria, and/or altered metabolic functioning [4]. While the exact mechanisms are not known, dysbiosis has been implicated in the pathophysiology of many disease states [4, 10], such as breast cancer [86], metabolic disease [87], and functional gut disorders [6, 88]. Dysbiosis can be observed at various taxonomic levels (i.e., phylum, class, genus, or species) [4]. The most predominant phyla residing in the intestines are reported to be *Bacteroidetes* and *Firmicutes*, followed by *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* [89]. Different genes are present and expressed at predictable levels by the species in each phyla, reflecting the physiological and metabolic contributions to human host health [90]. In theory, a GI tract with greater diversity expands the range of genes expressed by microbes and could allow more metabolic processes that occur in the GI tract to be outsourced to the microbiome, as well as contribute to the function and maturation of the CNS [91-93]. It is evident that the microbes residing in the gut contribute to many aspects of physical health, and has also been implicated in

mental health and mood disorders including anxiety and depression pathophysiology, symptoms, and treatment [94].

2.2.2 Mechanisms

The relationship between the gut microbiota, their metabolites, and the host immune system provides primary mechanisms by which dysbiosis contributes to disease development [10]. The immune system is primed in the first two years of life with the induction of the gut microbiome [95, 96]. Microbiota interact directly with the gut environment, including mucosal layers, epithelial cell surface receptors, immunologic molecules, and the lymphatic system [97-100], all which affect cytokine production, immune response, and inflammation [13]. The thick mucosal layer in the gut epithelium serves as a preventative barrier between the human body and gut contents. Notably, loss of mucus integrity can be initiated by pathogenic microbial colonization and can lead to the translocation of bacterial antigens [101]. Both commensal and pathogenic bacteria compete for colonization of mucosal layer to form polymicrobial communities [102]; however, whether these microbes produce their own biofilm contributing to healthy host mucosal barrier is still debated [103]. Because of the altered composition, metabolism, and mucosal integrity, dysbiosis has been associated with pathogenesis of both GI-related and non-GI-related disorders. Starting with induction and reinforcement of the immune system in infancy and through adulthood, the gut microbiome contributes to inflammatory response [96, 104]. Laboratory rodents born or bred without commensal microorganisms (e.g., germ-free) are known to have weak immune response and lower production of immune cells [96, 105]. Furthermore, the induction of the innate immune system can be triggered by the presence of pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) [106]. PAMPs and MAMPs are highly-conserved, essential structures for microbes, and are therefore presented on both pathogenic and non-pathogenic microorganisms

[107]. These structures are recognized by host cells via pattern recognition receptors (PRRs) and lead to an immune response cascade [106].

Gram-negative bacteria activate the immune system via lipopolysaccharide (LPS), which is typically attached to their cell wall [108]. The translocation of and release of LPS across the epithelial wall into the lymphatic system leads to LPS-associated toxicity [109], where LPS is known to induce the release of proinflammatory cytokines [110, 111]. In normal intestinal function, Gram-negative bacteria does not cross the epithelium [112]. However, with intestinal permeability, which occurs under stress conditions or in disorders like IBD, the tight junctions (TJ) loosen, consequently allowing larger molecules and microorganisms to cross the TJ barrier [113, 114].

Also contributing to host health, the gut microbiome has been shown to affect digestion and nutrient absorption [115], insulin sensitivity and secretion [116], and maintenance of gut barrier integrity [7]. Certain microbiota even provide nutrients via synthesis of vitamins, such as vitamin K and various forms of vitamin B [117]. Alternatively, some microbiota are able to ferment undigested carbohydrates and dietary fibers into short chain fatty acids (SCFAs), providing up to 10% of a human's daily energy requirements [115]. The phylum *Bacteroidetes* mainly produces acetate and propionate, while *Firmicutes* produce butyrate [118], and both phyla harvest energy from food that can be absorbed [119]. This extra energy harvest could be beneficial, in the way that SCFAs are thought to exert metabolic benefits [120-122], are a source of fuel for intestinal mucosa [123], and regulate gut hormones and appetite [124, 125]. On the other hand, greater levels of SCFA have been observed in obese individuals compared to lean counterparts [126] indicating the excess caloric harvest may be implicated in the pathophysiology of obesity. Traditionally, the ratio between *Firmicutes* and *Bacteroidetes* has been implicated in disease risk

and thought to be a hallmark of obesity [127]; however, the range of variability across studies and even individuals in recent research may diminish the relevance of this ratio [128, 129]. Propionate, acetate, and butyrate play a role in regulating hepatic glucose and lipid metabolism [130]. Further, butyrate is suggested to improve health by increasing insulin sensitivity, suppressing inflammation, and increasing expression of leptin, the appetite reducing adipocytokine [131-134]. With expanding research into the gut microbiome, it is not surprising that outcomes vary widely across studies and more questions arise than answers.

2.2.3 Modifying the Gut Microbiome

As a modifiable lifestyle factor, diet's impact on the gut microbiome has been thoroughly investigated [135]. Prolonged consumption of a high-fat diet has been linked to dysbiosis in both mice and humans [136]. Alternatively, a dietary pattern low in fat and high in carbohydrates can lead to an increase in Bacteroidetes [137], partly due to the presence of carbohydrate-metabolizing genes in this phylum and the increased availability of substrate. Microbial differences were observed following either an animal-based or plant-based diet, with accompanying metabolism changes in fermentation processes of carbohydrate and protein [40]. Dietary fiber is known to influence microbial composition, as some fibers can provide energy to commensal bacteria [138, 139]. Furthermore, food with pre- and probiotics have potential to alter microbial composition and improve health, however this avenue of research has not been thoroughly established in humans [140].

Another modifiable lifestyle behavior is PA or exercise. Exercise-induced alterations in the gut microbiome have been associated with immunity [141], body weight and metabolism [142], and anxiety and cognition [52], however, these studies were conducted in rodent models and the limited investigation in human populations consists of two known works. One study investigated

gut microbial changes following different exercise training regimens for 8 weeks, cardiorespiratory (CRE) or resistance training (RT). Individuals in the CRE group showed initial changes in the gut microbiome that were not maintained at the completion of the intervention period. Alternatively, RT participants showed no detectable changes in composition. The authors suggest that baseline microbial composition can predict exercise-associated gains [54]. The second study was an 8-week exercise intervention with three groups: exercise only, exercise with whey protein supplementation, and whey protein supplementation only. The authors observed modest changes in composition and function of the gut microbiome after increasing PA; however, these changes were not significant [53]. Further research is needed to understand the impact of exercise on the gut microbiome.

2.3 Mood Disturbance

2.3.1 Definitions, Prevalence, and Pharmacologic Treatments

Mood disturbance is characterized by feelings of distress, sadness, or symptoms of depression and anxiety, and can contribute to the development of mood disorders, such as bipolar disorder, MDD, and anxiety [58]. QOL encompasses physical, social, and mental well-being [59], and humans naturally experience stress and mood disturbance throughout the lifespan [143]. However, persistent mood disturbance can lead to emotional inflexibility and dysregulation as well as decreased QOL, which is a hallmark of mood disorders [60, 144]. It is estimated that one in five adults in the United States (US) will be affected by a mood disorder in their lifetime [145, 146] and these conditions are some of the leading causes of disability worldwide [147]. Individuals with mood disorders have a higher incidence of obesity [148], metabolic syndrome [149], and cardiovascular disease [150], as well as higher mortality rates from these conditions compared to the general population [62]. Therefore, QOL is severely impaired in patients with mood disorders

for a multitude of reasons [151]. Receiving a mood disorder diagnosis can be costly due to pharmacological and therapeutic interventions, more than doubling direct healthcare costs [152]. Additionally, indirect costs, such as absences from work or loss of productivity, are also associated with mood disorders and contribute to their burden [153, 154]. Common approaches to treatment of mood disorders are pharmacotherapy (i.e., medications) and psychotherapy (such as Cognitive Behavioral Therapy). While these are common methods for first-line treatment, engaging in one of these interventions only reduces symptoms in about two-thirds of patients [155]. Furthermore, medications are often accompanied with a wide array of side effects including bleeding abnormalities, hepatotoxicity, suicidality, and GI disturbances (nausea, vomiting, weight loss/gain, diarrhea, etc.) [156, 157]. Regardless of mood disorder diagnoses, psychological distress and altered mood can be seen in chronic diseases such as rheumatoid arthritis [158], breast cancer [159], HIV [160, 161], and diabetes [162]. Reducing the burden of mood disturbance in health and disease via integrative and alternative means is warranted.

2.3.2 Non-Pharmacological Treatments

Outside of pharmacological interventions, lifestyle improvements have shown efficacy in improving mood-related symptoms. An emerging factor that is suggested to reduce mood disturbance is diet. Dietary patterns and components have been linked to mood, anxiety, and depression [163-169]. Even in individuals without a diagnosed mood disorder, higher incidence of depression symptoms is associated with pro-inflammatory dietary patterns [170-172]. Evidently, even short-term adherence (i.e., 10 days) to a Mediterranean-style dietary pattern shows improvements in mood in young healthy adults [173, 174]. Dietary interventions have also been successful in reducing depression symptoms in a variety of populations including postmenopausal women [175], women with breast cancer [64], older community-dwelling adults [65], and free-

living individuals experiencing depressive symptoms [66]. Unfortunately, there have been limited dietary interventions to improve other mood disorders, such as anxiety or bipolar disorder [176].

A modifiable lifestyle factor that is known to improve mood is PA and exercise. In fact, a commonly accepted principle in the 1990s suggested just 20-40 minutes of aerobic exercise could improve anxiety and mood for several subsequent hours [177]. Substantial evidence exists to corroborate the beneficial effects of PA on mood and mental well-being [178-184]. Notably, it does not seem there is a single mechanism by which health benefits of PA are exerted, and there are several physiological and psychological adaptations that contribute to mental health improvements [63]. Proposed mechanisms for these improvements include enhanced acute inflammatory response [185, 186], decreased stress-induced cortisol response [187, 188], and improved aerobic capacity [189]. Exercise hypothetically functions to improve mood similarly to anti-depressant medications by increasing circulating serotonin and monoamines [68, 69, 190]. Improving diet quality and engaging in more PA would seemingly be successful in boosting mood. However, further research is needed to determine feasibility of these interventions in mood-altered populations who may already struggle to engage in optimal health behaviors.

2.3.3 Profile of Mood States

A common tool used to measure mood is the Profile of Mood States (POMS) questionnaire. The original 65-item survey was developed in the 1970s and measures six dimensions of mood, including 1) Tension/Anxiety, 2) Anger/Hostility, 3) Vigor/Activity, 4) Fatigue/Inertia, 5) Depression/Dejection, and 6) Confusion/Bewilderment [191]. In the 1990s, an additional seventh subscale was included, Self-Esteem Related Affect and the tool was abbreviated to 40 items. Individuals are asked to rate their current mood on a 5-point Likert scale ranging from “not at all” to “extremely”. A total mood disturbance score can be calculated by subtracting the positive

subscales (vigor and self-esteem related affect) from the combined negative subscales. Higher total scores indicate greater mood disturbance. POMS has been used to assess mood states in a variety of populations, including adolescents [192-194], athletes [195-197], type 1 diabetic patients [198], and physicians [199].

2.4 Gut-Brain Axis

2.4.1 Definitions and Preclinical Evidence

The gut microbiome has also been identified as a modifiable means to influence mood. The bidirectional communication between the gut microbiome and the CNS happens along the GBA. Germ-free animals have become a practical preclinical tool to explore the interaction between the gut microbiota and brain, behavior, and mood. In the absence of microbiota, germ-free animals display decreased serotonin receptors in the hippocampus [200], lowered anxiety behaviors [24], and transferrable depressive-symptoms from patients with MDD [25]. Antibiotic-induced dysbiosis in mice display decreased anxiety, altered neuromodulator production, and disrupted blood-brain barrier function, further supporting the GBA connection [26, 27, 201, 202].

2.4.2 Human Studies and Therapeutic Opportunities

In humans, intestinal dysbiosis has been linked to depression, anxiety, and elevated stress [94, 203], translating some preclinical studies. Functional gut disorders are frequently accompanied by stress, depression, and anxiety because of distorted interactions between the gut and the CNS [204]. Additionally, strong similarities in diversity and abundances of microbiota have been observed between patients with IBS and patients with depression [31]. It is estimated that approximately one-third and one-fourth of patients with IBD are affected by anxiety and depression symptoms, respectively [30]. In fact, IBS could be considered a stress-related brain-

gut-microbiome disorder [205]. This relationship is bidirectional in that a functional GI disorder can predict development of a mood disorder, and a mood disorder is predictive of functional GI disorder development [206]. Conversely, the gut microbiome and CNS communication is still altered in individuals without a functional gut disorder. For instance, two studies have shown an imbalanced Firmicutes to Bacteroidetes ratio in patients with MDD compared to controls [11, 207]. Causality has been displayed by several studies in which anxiety and depression symptoms were transmitted to rodents using FMT from anxious or depressed patients [23, 25, 28, 29, 208, 209]. There is current investigation into the efficacy of using FMTs in humans for the treatment of mood disorders, although most studies are under-powered. Several of these studies have proven successful in initially reducing depression symptoms [210-213], however, these results are not maintained long-term in all studies [214-216].,

One mechanism conferring benefit via FMT may be an increase in species producing SCFAs. Circulating SCFAs can strengthen the integrity of TJs at the blood brain barrier (BBB), limiting the metabolites and molecules that can enter the brain tissue [217]. Decreased TJ integrity permits microbial LPS to initiate an immune response and release of cytokines that can penetrate the BBB, resulting in altered neurological function, mood, and behaviors [218]. In addition, some bacteria within the gut can produce neurotransmitters and neuromodulators, such as serotonin, gamma-aminobutyric acid (GABA), acetylcholine, and catecholamines [219-222]. There are numerous mechanisms by which the gut microbiota influence mood via the GBA and along the CNS, providing multiple targets for therapeutic interventions.

An alternative therapeutic approach that has recently emerged is the modification of intestinal microbiota via probiotics or prebiotics [223]. Probiotics are microorganisms that beneficially contribute to host microbial flora when consumed, and prebiotics are nutrients that

feed specific bacterial species to influence gut microbial composition to confer host health benefit; the combination of probiotics and prebiotics is called a synbiotic [224]. Fructooligosaccharides, galactooligosaccharides and inulin are all prebiotics that can produce anxiolytic and antidepressant effects in preclinical models [225-227]. Similarly, probiotics exert reduction in depression and anxiety behaviors in rodents [228-231], although the species used in these studies widely vary. Human trials display mixed results on the efficacy of any -biotic intervention to improve mood disturbance, anxiety, or depressive symptoms in mood-altered individuals [232, 233] due to large heterogeneity between studies. Synbiotics may provide the most benefits of these -biotic treatments [234-236] because of the compounding effects. Pre- and probiotics are known to have minimal side effects [237] and these compounds may lead to other health benefits, such as reduced inflammation, improved GI function, and decreased immune response [238-242]. Maximum efficacy may be achieved if pre- and probiotics are administered with prescribed medications [243, 244], and may also reduce side effects of medications [245]. Ample preclinical evidence exists to suggest the gut microbiome can be manipulated for mood benefits, although translation into humans is currently unresolved and warrants more research.

2.5 Conclusion

The connection between the gut microbiome and mood is well-established. While chronic stress can alter microbial composition, it is proposed that the gut microbiome may be more stable during acute stress [246]. Regardless, a robust gut microbiome may equip individuals to cope with daily stress and ever-changing mood states. The compounding effect of diet and exercise on both mood and the gut microbiome open many avenues for non-pharmacological interventions in individuals with altered mood. However, these behaviors are not easily improved in individuals with high mood disturbance. Therefore, feasible interventions, such as pre- or probiotics, are

appealing and should be rigorously conducted. Although the current evidence is compelling, future research is needed to explore the relationship between the gut microbiome and mood disturbance to mitigate the burden of mood disturbance and disorders.

Chapter 3: Health-related quality of life is associated with fecal microbial composition in breast cancer survivors

3.1 Abstract

Purpose: To investigate relationships between body size, gut microbiome, and health-related quality of life (QOL) in breast cancer survivors (BCS) in a clinical trial. Methods: A cross-sectional substudy was conducted using baseline data from 70 BCS participating in a randomized controlled trial of a lifestyle intervention. Measures included anthropometrics, QOL (Short Form Health-related QOL Survey-36 [SF-36]), and 16S rRNA gene sequencing of fecal microbes. Participants were categorized by body mass index (BMI) into non-obese ($\leq$29.9 kg/m^2; n=38) and obese ($\geq$30.0 kg/m^2; n=32) groups. Differences in bacterial taxa between groups were assessed using Kruskal-Wallis one-way analysis of variance. Spearman and partial correlations explored associations between taxa and SF-36 subscales. Mediation analysis explored the relationship between BMI and SF-36 mental health summary score with alpha diversity as a mediator. Results: Most BCS (72.9%) were non-Hispanic white with average age of 61.6 ($\pm$8.7) years. Non-obese BCS had a significantly higher relative abundance of *Ruminococcus* (p=0.003), *Streptococcus* (p=0.049), *Roseburia* (p=0.035), and *Dorea* (p=0.003). No differences were observed for SF-36 subscales between groups. Physical Functioning, Vitality, and Mental Health subscales were negatively associated with *Ruminococcus* (ρ=-0.304, p=0.036; ρ=-0.361, p=0.012; ρ=-0.495, p<0.001) and *Dorea* (ρ=-0.378, p=0.028; ρ=-0.33, p=0.022; ρ=-0.388, p=0.006) abundance controlling for BMI. Conclusions: Fecal microbial composition differed between obese and non-obese BCS, with associations between QOL and several microbial taxa. Several of these genera, previously identified as potentially beneficial may also influence QOL in BCS. These results

support further studies to determine the role of individual microbiota in QOL and obesity in cancer survivors.

3.2 Introduction

The population of cancer survivors is increasing in the United States (US), with an estimated 22.1 million survivors by the year 2040 [247]. The National Cancer Institute (NCI) reports breast cancer represent 15% of new female cancer cases in the US [248]. Additionally, the NCI estimates 90.6% of females with breast cancer survive 5-years or more past diagnosis [248]. As compared to individuals without a cancer history, older cancer survivors experience a greater decline in physical functioning and health-related quality of life (QOL), and are more prone to higher levels of anxiety, depression, and fatigue [249, 250]. Breast cancer survivors (BCS) with obesity exhibit lower mental QOL compared to non-obese BCS, as well as controls without breast cancer [251]. Moreover, it is estimated that roughly 60% of women gain weight following a breast cancer diagnosis [252]. Disease-related weight gain could be attributed to chemotherapy side effects [253], decreased physical activity (PA) [254], or being post-menopausal or becoming post-menopausal after treatment [255]. This weight gain can be mentally distressing [256] and may also lead to decreased survival [257-259].

Known to modulate human host health, intestinal microbiota communicate bidirectionally with the CNS through various pathways along the gut-brain axis (GBA). Recent research shows the GBA can influence human host behavior, cognition, and emotions [260] which affects overall health and QOL. Reciprocally, fear of cancer recurrence has been associated with decreased microbial diversity in BCS [261]. Gut dysbiosis is defined as an imbalance of detrimental microbes at the expense of commensal microbes [87, 250] and is associated with increased systemic inflammation, risk and progression of various cancer types, and obesity-related metabolic

dysfunctions [262-264]. Cancer survivors often display gut dysbiosis from chemotherapy, hormone therapy, or radiation side effects [261, 265, 266] and/or poor dietary habits [267]. Gut dysbiosis has also been suggested to play a role in cancer recurrence, although these mechanisms are not clearly understood [268].

The purpose of this study was to investigate the relationships between body mass, the gut microbiome, and health-related QOL in BCS using cross-sectional baseline data from a previously conducted trial. We also sought to determine how differences in the microbiome can affect overall QOL and the role of certain microbiota in QOL for cancer survivors with varying body size.

3.3 Methods

This is a cross-sectional analysis of a previous wait-listed randomized controlled trial with a vegetable gardening intervention; only relevant study materials and methods are included. Detailed methods of this trial have been reported [269, 270] and are briefly described herein.

3.3.1 Participants

Potential participants identified from the Alabama Statewide Cancer Registry and individual hospital registries were mailed recruitment invitations. Interested BCS were then screened for the following inclusion criteria: 1) conclusion of cancer treatment; 2) currently eating <5 servings of fruits and vegetables per day; 3) exercising <150 minutes per week; 4) ≥1 physical function limitation; 5) ability to speak and write English; 6) residing within ≤15 miles of Master Gardener; 7) residence with ≥6 hours of sun per day, running water, and accommodation for gardening beds or boxes; 8) willingness for randomization to either study group. BCS were excluded on for the following criteria: 1) comorbid conditions that would impair the individual's ability to complete study assessments or participate in supervised PA; 2) current use of pharmacological doses of warfarin; or 3) having tended a successful vegetable garden within the

past 2 years. Eligible and interested participants were allocated to either the intervention or wait-list control group after baseline data collection.

3.3.2 Data Collection

Health-related QOL was assessed using the Medical Outcomes 36-item Short-Form Health Survey (SF-36) self-report questionnaire [271]. The SF-36 commonly used to measure both physical and mental health within 8 subscales (physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, mental health) and 2 summary domains (physical component summary and mental component summary scores).

Anthropometric measurements included height and weight using portable stadiometer and calibrated scale, respectively, while the participant was in light clothing and without shoes. These measurements were used to determine body mass index (BMI). Participants were categorized by BMI into obese ($\geq$30.0 kg/m^2) and non-obese ($\leq$29.9kg/m^2) categories for between group comparisons.

Sterile wipes were provided to collect stool samples. Participants used the sterile wipe after a bowel movement, placed the wipe in a sealed plastic bag, and immediately stored the wipe in their freezer after noting the date and time of the collection. Study staff collected samples from participants at home visits and stored them at -80°F until processing. Microbial DNA was extracted from the samples, the V4 region of the 16s rRNA V4 gene was PCR-amplified and then sequenced using Illumina Miseq [272, 273]. The Quantitative Insight into Microbial Ecology (QIIME, suite version 1.7) was used to determine operational taxonomic units (OTUs). Full details of stool sample processing have been described previously [272].

3.3.3 Statistical Analysis

Descriptive statistics were obtained and obese (n=32) and non-obese (n=38) groups were compared using independent t-tests for continuous variables and chi-square tests for categorical variables. Certain genera were selected for this analysis based on literature indicating previous associations with cancer, obesity, and/or mental health. Relative abundance of these genera was used to compare obese and non-obese groups using Kruskal-Wallis one-way analysis of variance. Alpha-diversity was determined using Chao1, Observed Species, Whole Tree Phylogeny, and Shannon and Simpson indices and differences between groups were compared using independent samples t-tests. Associations between bacterial taxa at the genus level and SF-36 subscales were explored using Spearman correlations within groups and partials correlations for all participants controlling for BMI. Statistical significance threshold was set at $p<0.05$.

3.3.3.1 Mediation Analysis

To investigate how observed species mediated the relationship between BMI and Mental Component Summary score, a simple mediation analysis was performed using PROCESS [274]. The outcome variable was SF-36 mental component summary score (MCS), the predictor variable was BMI, and the mediator was alpha diversity, as measured by Total Observed Species. Race, age, and treatment type were used as covariates in the model to account for potential differences in these variables. Due to the significant effects of chemotherapy on the gut microbiome [265], treatment was dichotomized into those that received chemotherapy (n=45) and those that did not receive chemotherapy (n=25). Post-hoc independent samples t-test showed no significant differences in Observed Species or MCS. Mediation analysis tested the indirect effect of the mediator (total observed species) on the relationship between the predictor (BMI) outcome variable (MCS). In SPSS version 25, the PROCESS macro version 4.0 [274] utilizing model 4

with 95% confidence interval was used to investigate the hypothesized model (see Figure 3.1). Significant effects are supported by the absence of zero within the confidence intervals.

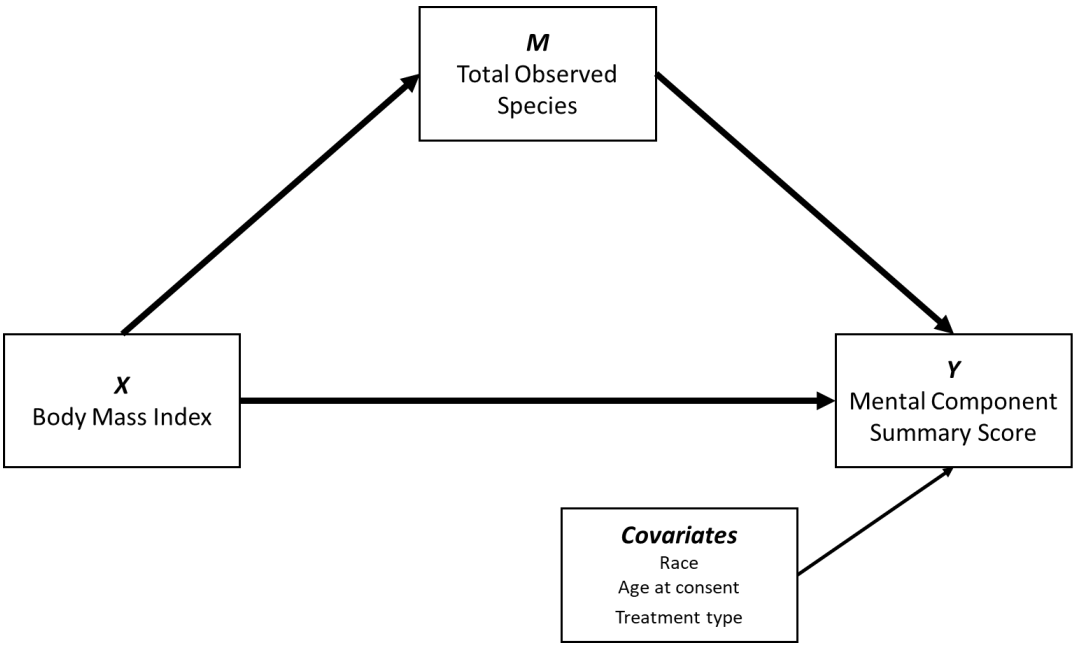

Figure 3.1. Hypothesized simple mediation model of Body Mass Index (X) on Mental Component Summary score of health-related quality of life (Short Form-36) with Total Observed Species as a mediator. Race, age at consent, and treatment type are covariates controlled for in this model. (P-value = 0.275)

3.4 Results

Table 3.1 presents characteristics of the study sample. Participants were mostly non-Hispanic white (72.9%) and aged 61.6 years on average. There were no differences in any SF-36 subscales between obese and non-obese BCS. Most participants received chemotherapy (65.2%) or radiation (63.8%), with no significant differences in treatment between obese and non-obese BCS.

Table 3.1. Demographics of female breast cancer survivors enrolled in a clinical trial by BMI groups

	All (n=70)	Non-Obese (n=38)	Obese (n=32)	p-value
	Mean (SD)			
Age (years)	61.6 (8.7)	61.2 (7.1)	62.1 (10.3)	0.667
Body Mass Index (kg/m^2)	30.4 (6.7)	25.8 (3.3)	35.8 (5.5)	<0.001
Short Form-36 Subscales				
Physical Functioning	68.7 (22.2)	72.5 (19.8)	63.8 (24.4)	0.110
Role Physical	70.1 (27.5)	67.8 (28.8)	72.9 (26.1)	0.445
Bodily Pain	62.6 (21.9)	62.6 (23.7)	62.7 (20.0)	0.996
General Health	62.6 (21.3)	65.0 (21.4)	59.8 (21.1)	0.316
Vitality	51.2 (19.9)	52.6 (21.5)	49.4 (18.0)	0.507
Social Functioning	76.6 (24.7)	76.3 (26.1)	77.0 (23.4)	0.915
Role Emotional	79.6 (22.6)	79.6 (24.4)	79.7 (20.7)	0.988
Mental Health	74.9 (18.5)	74.3 (19.9)	75.6 (16.9)	0.775
Physical Component Score	65.9 (19.4)	67.0 (19.8)	64.5 (19.1)	0.601
Mental Component Score	70.4 (18.9)	70.7 (20.7)	70.0 (16.9)	0.874
	N (%)			
Race				1.000
Black	19 (27.1)	10 (26.3)	9 (28.1)	
White	51 (72.9)	28 (73.7)	23 (71.9)	
Chemotherapy				0.206
Yes	45 (65.2)	27 (73)	18 (56.3)	
No	24 (34.8)	10 (27)	14 (43.8)	
Radiation Therapy				0.461
Yes	44 (63.8)	22 (59.5)	22 (68.8)	
No	25 (36.2)	15 (40.5)	10 (31.3)	
Hormone Therapy				0.994
Yes	28 (40.0)	15 (39.5)	13 (40.6)	
No	41 (58.6)	22 (57.9)	19 (59.4)	

Table 3.2 reports relative abundance of relevant bacterial taxa by BMI group. Non-obese BCS had a significantly higher relative abundance of *Ruminococcus* (p=0.003), *Streptococcus* (p=0.049), *Roseburia* (p=0.035), and *Dorea* (p=0.003). BCS with obesity had a higher relative abundance of *Pseudomonas* (p=0.016) and *Proteus* (p=0.017). There were no significant

differences between alpha diversity measures between obese and non-obese BCS, which are reported in Table 3.3.

Table 3.2. Kruskal-Wallis ANOVA assessment of differences in bacterial taxa between body mass index groups

Genus	All (n=70)	Non-Obese (n=38)	Obese (n=32)	*p*-value
Blautia	0.1216	0.1425	0.0942	0.290
Acinetobacter	0.1678	0.1811	0.1504	0.216
Escherichia	0.0182	0.0209	0.0146	0.227
Bacteroides	0.0717	0.0559	0.0924	0.121
f_Enterobacteriaceae;g_Other*	0.0205	0.0250	0.0147	**0.035**
Faecalibacterium	0.0554	0.0516	0.0602	0.483
Tissierella_Soehngenia	0.0568	0.0886	0.0152	0.060
Ruminococcus	0.0307	0.0255	0.0375	0.732
Coprococcus	0.0253	0.0279	0.0220	0.602
Akkermansia	0.0241	0.0132	0.0382	0.917
Prevotella	0.0193	0.0172	0.0220	0.612
Pseudomonas#	0.0091	0.0048	0.0146	**0.016**
*[Ruminococcus]**	0.0186	0.0230	0.0128	**0.003**
*Streptococcus**	0.0209	0.0308	0.0080	**0.049**
*Roseburia**	0.0222	0.0283	0.0143	**0.035**
f__Lachnospiraceae;g_	0.0175	0.0204	0.0137	0.170
f_Ruminococcaceae;g_Other	0.0161	0.0177	0.0140	0.152
f_Lachnospiraceae;g_Other	0.0158	0.0189	0.0117	0.084
Bifidobacterium	0.0088	0.0129	0.0035	0.161
Lactobacillus	0.0116	0.0180	0.0032	0.107
Peptoniphilus	0.0127	0.0133	0.0120	0.194
Oscillospira	0.0094	0.0095	0.0093	0.257
Enterococcus	0.0015	0.0020	0.0009	0.709
*Dorea**	0.0089	0.0119	0.0049	**0.003**
Proteus#	0.0002	0.0002	0.0002	**0.017**
Clostridium	0.0081	0.0108	0.0045	0.748
Lachnospira	0.0020	0.0015	0.0026	0.122
*Fusobacterium**	0.0062	0.0083	0.0035	**0.019**
Sutterella#	0.0024	0.0012	0.0038	**0.020**
*#higher in obese group; *higher in non-obese*				

Table 3.3. Alpha Diversity metrics by obese and non-obese groups of breast cancer survivors after completion of treatment

	All (n=70)	Non-Obese (n=38)	Obese (n=32)	*p*-value
Chao1	377.7 (60.3)	367.9 (66.6)	390.5 (49.3)	0.152
Observed Species	300.1 (60.0)	294.2 (67.6)	307.8 (48.6)	0.390
Phylogenetic Diversity Whole Tree	20.7 (3.1)	20.5 (3.2)	21.0 (3.0)	0.492
Shannon Index	4.6 (1.3)	4.4 (1.3)	4.9 (1.2)	0.145
Simpson Index	0.8 (0.2)	0.8 (0.2)	0.9 (0.1)	0.243

Partial correlations controlling for BMI are reported in Figure 3.2. *Ruminococcus* was negatively associated with the following SF-36 subscales: Physical Functioning (ρ=-0.304, p=0.036), Vitality (ρ=-0.361, p=0.012), and Mental Health (ρ=-0.495, p<0.001). The genus Dorea was also negatively correlated with Mental Health (ρ=-0.388; p=0.006), as well as Social Functioning (ρ=-0.373; p=0.009), and mental component summary score (ρ=-0.393; p=0.006). The effect sizes of significant correlations were less than 0.5 and therefore reflected low to moderate effects [275].

Results from the simple mediation analysis indicate observed species did not significantly mediate the effect of BMI on MCS (b= -0.48, t(53)= -1.104, p=0.275). With race as a covariate, the model was improved (b= -12.71, t(53)= -2.19, p=0.033). However, addition of the remaining covariates (age at consent and chemotherapy treatment) did not improve the ability to predict MCS through total observed species. The indirect effect of BMI on MCS was not statistically significant (Indirect effect = 0.064, SE 0.119, 95% CI [-0.106, 0.384]). Therefore, BMI was directly and negatively associated with MCS.

3.5 Discussion

To our knowledge, this is the first study to investigate the relationship between QOL and the gut microbiome in BCS. We sought to determine the relationship between QOL and fecal

microbial composition among obese and non-obese BCS. We also noted several differences in intestinal bacterial taxa between the BMI groups. Several associations between QOL and microbial taxa were observed among this sample of BCS.

Obesity has been linked to worsened breast cancer outcomes, including large tumors, greater risk of metastasis, and increased mortality [276]. Connor et al. found BMI was associated with lower mental health sub-scores in BCS, however, this result was not observed in the present study. While our study recruited BCS after completion of treatment, regardless of length of time, Connor et al. exclusively recruited long-term survivors with an average 14.5 years since date of diagnosis [251]. Furthermore, sample populations differed in race and ethnicity composition, where Connor et al. exclusively analyzed Hispanic and Non-Hispanic White BCS, and the present study included Black and White BCS. Lastly, the BCS included in our study were slightly younger on average (mean age = 61.6 years; SD = 8.7 years) compared to the study by Connor et al. (mean age = 64.3 years; SD = 9.1 years). Differing results could be attributed to the long-term follow up, suggesting higher BMI may reduce mental health recovery or lower BMI individuals have better rebound of mental health in the long-term years after a breast cancer diagnosis. This could also suggest that age plays a critical role in mental health resilience in BCS, although these factors need further investigation.

On the other hand, certain bacterial genera and/or species are implicated in mental health conditions and mood disorders. The genus *Bacteroides* was shown to be elevated in patients with generalized anxiety disorder [12], and patients experiencing an anxiety or depressive episode have an over-represented abundance of *Bacteroides* [277]. Another study conducted by Okubo et al. investigated how the concerns of cancer recurrence in BCS are related to the composition of the gut microbiome. In these Japanese BCS, *Bacteroides* abundance was positively associated with

fear of cancer recurrence, while *Lachnospiraceae.g* was negatively associated with this fear [261]. In the present study, we found no association between SF-36 sub-scales and *Bacteroides* genus after controlling for BMI. Concurrent with Okubo's findings, there were no associations between any SF-36 subscales or summary scores and abundance of several genera (e.g., *Faecalibacterium*, *Blautia*, and *Coprococcus*). However, we observed negative correlations between *Lachnospiraceae.g* and Social Functioning, and Mental Health subscales, and the Mental Component Summary score. This bacteria belongs to the Firmicutes phylum, which has been shown to be severely diminished following chemotherapy treatment [265].

Short-chain fatty acids (SCFA) are produced during the fermentation of non-digestible carbohydrates by certain bacterial genera. *Ruminococcus* and *Roseburia* are both known to produce the SCFA butyrate [130, 278, 279] while *Streptococcus* and *Dorea* produce acetate [280]. SCFAs are essential for colonocytes, as they are utilized for energy by these epithelial cells [281] but are also known for their benefits in immune function, carcinogenesis inhibition, epithelial barrier function, and satiety [280]. *Ruminococcus*, *Roseburia*, *Streptococcus*, and *Dorea* were observed to be in higher abundance in non-obese BCS in the present study, thus supporting their contributions to host health.

Conversely, BCS with obesity had higher abundance of *Proteus* and *Pseudomonas* genera. *Proteus,* although present in low abundance in the human gut, has been identified in the pathophysiology of GI disease, specifically Crohn's disease [282]. Moreover, some species of the genus *Pseudomonas* are known opportunistic pathogens in humans and have been found in high abundance in multiple sclerosis patients [283].

3.5.1 Limitations

The results herein add to our understanding of the relationship between the fecal microbiome and QOL, however, there are limitations to this research. First, we did not collect participants' bodyweight pre-diagnosis or before initiation of therapy, thus the effects of treatment on their weight at the point of data collection could not be explored. Likewise, fecal samples were not collected at an earlier timepoint, and therefore, we were not able to observe changes to the gut microbiome following treatment. Similarly, the observational nature of this cross-sectional study and our mediation analysis precludes assessing causality or directionality in the relationships observed. Cancer treatment methods were not homogenous among participants, with 63.8% of participants receiving radiation treatment. Radiation and chemotherapy treatment are both known to cause dysbiosis [261] and these compositional changes may influence the genera present, although it would affect each participant differently. The use of sterile wipes instead of solid stool collection may introduce bacteria not present in the GI tract, though recent evidence suggests this method is comparable to this standard method with regards to bacterial abundance and diversity [284]. Finally, although the sample was racially diverse, the women resided exclusively in the greater Birmingham area of Alabama, which may not be generalizable to the larger US population.

3.5.2 Conclusions

Obese and non-obese BCS displayed differing compositions of fecal microbiota. Several microbial genera were associated with vitality, social functioning, and mental health. Additionally, alpha diversity was not a mediator of the relationship between BMI and mental QOL. Further investigation is warranted to understand the role of the gut microbiome in obesity and health-related QOL in the ever-growing population of BCS.

Figure 3.2. Partial correlations heatmap between Genus and Short-Form 36 Subscales after controlling for Body Mass Index

Genus	Physical Functioning	Role Physical	Bodily Pain	General Health	Vitality	Social Functioning	Role Emotional	Mental Health	Physical Component Summary	Mental Component Summary
Blautia	0.031	-0.015	0.168	0.128	0.205	-0.038	0.081	0.047	0.089	0.075
Acinetobacter	0.219	0.01	**-0.293**	-0.018	-0.007	0.139	0.054	0.033	-0.029	0.065
Escherichia	-0.014	0.105	0.093	0.074	0.194	0.06	0.055	0.126	0.08	0.115
Bacteroides	-0.069	0.028	0.019	0.022	-0.002	0.004	0.152	0.05	0.003	0.057
f_Enterobacteriaceae;g_Other	**-0.318**	-0.226	-0.143	**-0.402**	-0.221	-0.264	**-0.298**	**-0.294**	**-0.319**	**-0.297**
Faecalibacterium	-0.185	-0.023	0.022	-0.126	-0.029	-0.056	-0.052	-0.057	-0.086	-0.054
Tissierella_Soehngenia	-0.167	-0.156	-0.11	-0.206	-0.267	-0.236	-0.279	-0.262	-0.189	**-0.287**
Ruminococcus	-0.18	-0.096	0.119	0.027	0.004	0.02	-0.001	0.16	-0.038	0.047
Coprococcus	-0.132	-0.087	0.047	-0.096	-0.058	-0.191	-0.126	-0.106	-0.079	-0.137
Akkermansia	0.079	0.099	0.027	-0.017	0.232	0.119	0.138	0.142	0.058	0.171
Prevotella	0.199	0.157	0.01	-0.026	-0.029	0.013	0.159	-0.065	0.103	0.026
Pseudomonas	-0.031	-0.134	-0.21	-0.276	-0.051	-0.024	-0.124	0.042	-0.194	-0.046
[Ruminococcus]	**-0.304**	-0.229	-0.052	-0.236	**-0.361**	**-0.502**	**-0.383**	**-0.495**	-0.242	**-0.482**
Streptococcus	-0.012	0.108	0.152	-0.054	0.132	0.128	0.166	0.08	0.062	0.141
Roseburia	-0.088	0.01	-0.023	-0.02	0.016	-0.158	-0.104	-0.105	-0.033	-0.101
f__Lachnospiraceae;g_	-0.256	-0.203	-0.12	-0.102	-0.202	**-0.365**	-0.239	**-0.36**	-0.202	**-0.324**
f_Ruminococcaceae;g_Other	-0.05	0.149	0.251	0.137	0.218	0.223	0.143	0.241	0.15	0.226

f_Lachnospiraceae;g_Other	-0.241	**-0.307**	-0.105	0.154	-0.217	-0.256	-0.227	-0.218	-0.156	-0.255
Bifidobacterium	0.141	0.138	-0.07	0.023	0.111	0.067	0.098	-0.003	0.071	0.077
Lactobacillus	0.199	0.098	0.088	-0.123	-0.081	0.155	0.166	0.066	0.078	0.092
Peptoniphilus	0.076	-0.069	-0.12	0.065	-0.154	-0.072	0.009	-0.162	-0.02	-0.099
Oscillospira	-0.153	0.078	0.099	0.011	0.013	0.119	0.07	0.104	0.017	0.086
Enterococcus	0.093	0.185	0.192	0.095	0.257	0.047	0.099	0.141	0.171	0.144
Dorea	**-0.318**	**-0.308**	-0.199	-0.187	**-0.33**	**-0.373**	**-0.335**	**-0.388**	**-0.302**	**-0.393**
Proteus	0.09	0.193	-0.058	0.101	0.074	-0.073	0.016	-0.056	0.101	-0.013
Clostridium	-0.086	-0.18	-0.037	-0.093	-0.094	-0.086	-0.182	-0.164	-0.122	-0.144
Lachnospira	-0.208	-0.045	0.211	0.068	0.146	0.22	0.063	0.229	0.01	0.181
Fusobacterium	-0.146	-0.016	-0.173	-0.06	-0.036	-0.128	-0.198	-0.149	-0.112	-0.143
Sutterella	-0.117	0.105	0.198	0.175	0.179	0.21	0.12	0.165	0.112	0.187

Heatmap indicates degree of association between abundance of genus and SF-36 subscales after controlling for BMI; blue represents positive correlations and red represents negative correlations; bold values indicate significant association ($p<0.05$)

Chapter 4: Mood disturbance is associated with fecal microbiome diversity in free-living adults

4.1 Abstract

Purpose: Gut microbiota are known to modulate neurotransmitters, affecting the host's mental state. Because diet directly impacts gut microbial composition, we aimed to explore the relationship between mood disturbance (MD), diet quality (DQ), and the fecal microbiome in free-living adults. Methods: A cross-sectional analysis was conducted with baseline data from 75 healthy adults enrolled in two separate studies. Measures included anthropometrics, 16s rRNA gene sequencing of fecal microbes, DQ as assessed by Healthy Eating Index-2015 (HEI), and MD determined by the abbreviated Profile of Mood States (POMS). Participants were dichotomized at the median into low (n=37) and high MD (n=38) to explore alpha diversity and DQ differences in these groups. Spearman correlations were used to investigate relationships between alpha diversity, DQ, and POMS subscales. Moderation analysis was used to explore the effect of HEI score on the relationship between MD and alpha diversity. Results: Participants were mostly white (67%), 54.5 years old (±11.8), and overweight (body mass index 28.5 ± 6.5 kg/m^2). Shannon and Simpson indices indicate higher alpha diversity in participants with low MD compared to high MD (p=0.004 and p=0.008, respectively). Simpson and Shannon indices were positively correlated with the anger subscale (rho= -0.303, p=0.011; rho= -0.265, p=0.027, respectively) and total MD (rho= -0.404, p=0.001; rho= -0.357, p=0.002, respectively). Refined grains were associated with fatigue and tension subscales (rho=0.428, p<0.001; rho=0.302, p=0.014, respectively). Moderation results indicate the moderating effect of DQ on the relationship between alpha diversity and MD in healthy adults was not significant ($F(7, 53) = 2.00$, p=0.072, R^2=0.209). Shannon index was a significant predictor of MD (b= -4.39, t(53)= -2.55, p=0.014), but total HEI score (b=0.096, t(53)=0.66, p=0.515) and the interaction (Shannon index*HEI score) were not significant (b= -

0.01, t(53)= -0.09, p=0.931). Conclusions: Greater bacterial diversity was associated with lower MD, and DQ was associated with various mood state subscales in this sample of adults.

4.2 Introduction

Mood disorders include major depressive disorder (MDD), obsessive-compulsive disorder, anxiety disorder, and bipolar disorder that affect one in five adults in the US during their lifetime [44] and influence the individual's emotional state, energy and motivation [58]. These conditions are among the leading causes of disability across the world [147], and a mood disorder diagnosis more than doubles direct healthcare expenses via psychological and pharmaceutical treatment [152]. Medications are accompanied by a range of side effects including heat intolerance, sexual dysfunction, dry mouth, and a variety of GI disturbances (i.e., nausea, diarrhea, constipation, and indigestion) [156, 157]. Because of the economic burden and adverse effects of treatment, supportive care, and alternative/integrative therapy for individuals with mood disorders is increasingly important.

The gut-brain axis (GBA) describes the bidirectional interaction between the GI tract and the brain [285] and impacts both the pathophysiology and treatment of mood disorders [94, 286]. More specifically, the microorganisms residing in the GI tract have been found to play an integral role in mental and physical health, as well as host homeostasis [260, 287]. Substantial pre-clinical evidence demonstrates that the gut microbiome influences brain function and behavior [288], and preliminary evidence supports this relationship in humans as well [288, 289], with the gut microbiome implicated in the etiology of a variety of mood-related conditions [290, 291]. Patients with MDD or generalized anxiety disorder display different fecal microbiome profiles compared to healthy controls [11, 12]. A recent systematic review estimates that 35% and 22% of patients with inflammatory bowel disease experience anxiety or depressive symptoms, respectively [33].

The Profile of Mood States (POMS) questionnaire is a common tool used to measure mood and assesses transient, distinct mood states, including depression and anxiety, that contribute to overall mood disturbance [191]. Thus, assessing mood disturbance may serve as a proxy for subclinical mood disorders.

Environmental factors such as diet can greatly impact the composition of the gut microbiome [292] and the etiology of mood and cognitive disorders [293, 294]. Consumption of non-digestible carbohydrates (i.e., dietary fiber) can aid in the proliferation of favorable gut microbiota, leading to rapid microbial composition shifts [295] [292]. Dietary patterns rich in non-digestible carbohydrates and unsaturated fats, such as the Mediterranean diet, are associated with reduced depression risk [163, 296], while overconsumption of refined carbohydrates or trans fats is associated with increased depressive symptoms [297]. Thus, dietary factors may moderate the interaction of the gut microbiome and mood disorder symptoms. Herein, we investigated the relationship between gut microbiome composition and mood disturbance in free-living adults and explored the moderating effect of DQ on this relationship.

4.3 Methods

We conducted a cross-sectional analysis of participants enrolled in a randomized controlled trial (NCT 04015479), Peanut Protein Supplementation study (PPS), and the Diet, Inflammation, and Microbes study (DIM). Relevant study methods are described below. Both study protocols were approved by the Institutional Review Board at Auburn University (PPS: Protocol # 19-249 MR 1907 and DIM: Protocol # 18-285 EP 1807). Participants submitted informed written consent prior to commencement of data collection. All procedures were in accordance with the Declaration of Helsinki.

4.3.1 Participants

4.3.1.1 Peanut Protein Supplementation Study

This study examined the effects of resistance training (RT) on muscle mass and strength in untrained older adults. Expansive methods have been previously described [298]. Participants were recruited and enrolled from August 2019 to January 2020. Eligibility criteria included: 1) aged 50-80 years old; 2) not actively participating in structured RT for at least three months prior; 3) free of metal implants; and 4) blood pressure (BP) readings that were indicative of being less than stage 2 hypertension (with or without medication; i.e., less than 140/90 Systolic BP/Diastolic BP). Participants were ineligible for the following reasons: 1) known peanut allergy; 2) body mass index (BMI) >/= 35 kg/m^2; 3) exposure to medically necessary radiation in the last six months; and 4) contradictory medical condition that prevents individual participating in a RT program or any of the testing procedures, such as donation of blood or skeletal muscle biopsy (i.e., blood clotting disorders or taking blood thinning medications). Data obtained at baseline is included in the current analysis.

4.3.1.2 Diet, Inflammation, Microbes Study

This study enrolled individuals not meeting eligibility criteria for a dietary intervention randomized controlled trial (NCT03582306) [299], the Meat and Three Greens Feasibility Trial (M3G). Participants were recruited and enrolled from July 2018 to September 2018. Individuals were screened for eligibility prior to consent using an online questionnaire designed to capture habitual red meat and green leafy vegetable consumption and self-reported anthropometric information. M3G eligibility included high red meat intake, low green leafy vegetable intake, and BMI > 30 kg/m^2. Those not meeting M3G requirements were offered enrollment to the DIM cross-sectional study.

4.3.2 Data Collection

4.3.2.1 Anthropometrics

Height and weight were measured using laboratory scales, rounded to the nearest 0.1kg and 0.5cm, and were used in the calculation of BMI. In the PP study, body composition was assessed with a full-body dual energy x-ray absorptiometry (DEXA) to estimate soft lean tissue mass and fat mass. In the DIM study, body composition was determined using a handheld body impedance analysis (BIA) device (Omron HBF-306C, Omron Healthcare, Inc. Lake Forest, IL). Research suggests BIA is a suitable alternative to DEXA for individuals within normal weight and body fat percentage ranges [300].

4.3.2.2 Fecal Sample Processing

Fecal sample collection was identical for both studies. Samples were collected using a commode specimen collector and sterile collection tube. Immediately following collection, participants stored fecal sample in their freezer until submission at next appointment. Submitted samples were stored at -80°C prior to processing. Microbial DNA within the fecal sample was isolated using Zymo Research kits (Irvine, CA, USA). Isolated DNA was prepared before polymerase chain reaction (PCR) amplified the 250 base pair variable region 4 of 16S rRNA. The PCR library was sequenced using the Illumina Miseq (San Diego, CA, USA), and informatic analysis was conducted using the Quantitative Insight into Microbial Ecology (QIIME) suite, version 1.7 using DADA2 as previously described [301, 302]. Alpha diversity was measured using Observed Species, Whole Tree Phylogeny, and Shannon and Simpson indices. Abundances of the top ten genera were used in the analysis.

4.3.2.3 Dietary Analysis

In the PP study, participants were instructed to maintain current dietary habits and record food intake for 3 consecutive days after a brief training by study staff. Upon receipt of food logs, study staff entered records into the Automated Self-Administered 24-hour Dietary Recall (ASA-24) software, which utilizes the US Department of Agriculture Food and Nutrient Database for Dietary Studies that provides values for 195 nutrients and food components [303]. Participants in the DIM study were contacted by a Registered Dietitian (RD) or dietetics student who administered the two 24-hour dietary recalls and entered the collected information into ASA-24. Dietary recalls were collected on one weekday and one weekend day prior to baseline appointments. For both studies, each participant's calorie and macronutrient intake represents the average of all days in which data were collected.

The Healthy Eating Index-2015 (HEI) was designed to measure adherence to the 2015 Dietary Guidelines for Americans and is widely used to assess DQ [304]. The HEI yields component scores for total fruits, whole fruits, total vegetables, greens and beans, whole grains, dairy foods, total protein foods, seafood and plant proteins, fatty acids, refined grains, sodium, added sugars, and saturated fats. These data from ASA-24 output were utilized to evaluate HEI component scores, which were calculated based on ratios per 1,000 kilocalories and assigned values based on scoring standards. These component scores were totaled for an overall HEI score ranging from 0-100, with higher scores indicating greater adherence to dietary guidance and healthier eating habits.

4.3.2.4 Mood Disturbance

Mood disturbance (MD) was assessed using the abbreviated Profile of Mood States (POMS) instrument [305]. This 40-item questionnaire measures seven psychological distress and

mood dimensions: 1) tension/anxiety; 2) anger/hostility; 3) vigor/activity; 4) fatigue/inertia; 5) depression/dejection; 6) confusion/bewilderment; 7) esteem-related affect. The vigor/activity and esteem-related affect subscales are considered positive subscales, while the remaining five subscales are negative in relation to MD. Individuals are asked to rate their mood using a 5-point Likert scale ranging from "not at all" to "extremely". Total MD is calculated by subtracting the total positive subscales (vigor + esteem-related affect) from the total negative subscales and adding a constant (100) to eliminate negative scores. A higher score indicates greater MD.

4.3.3 Statistical Analysis

All statistical analyses were conducted using SPSS version 25.0 (IBM Corp, Armonk, NY, USA). Spearman correlations determined associations between alpha diversity metrics, individual microbes, POMS subscale domains, and HEI components. Participants were dichotomized at the median (86) into low and high MD to explore differences in HEI components, alpha diversity, and genera abundance. Independent samples t-tests were used for comparison of alpha diversity and HEI components between low and high MD. Kruskal-Wallis one-way analysis of variance tests were performed to compare relative abundance of the top ten genera with false discovery rate (FDR) corrections. Data are presented as mean ± standard deviation (SD) values throughout.

4.3.3.4 Moderation Analysis

A simple moderation analysis was performed using PROCESS macro version 4.0 in SPSS v25 to investigate how diet moderates the relationship between alpha diversity metrics and MD in healthy adults. The outcome variable was MD, the predictor variable was alpha diversity as measured by Shannon Index, and the moderator was the total HEI score. To account for confounding differences, age, race, sex, and BMI were used as covariates in the model. The

PROCESS macro version 4.0 in SPSS version 25.0 was utilized for the analyses [274], using model 1 with 95% confidence interval to explore the hypothesized model (Figure 4.1). The Johnson-Neyman technique was used to explore the regions of significance for conditional effects of the model.

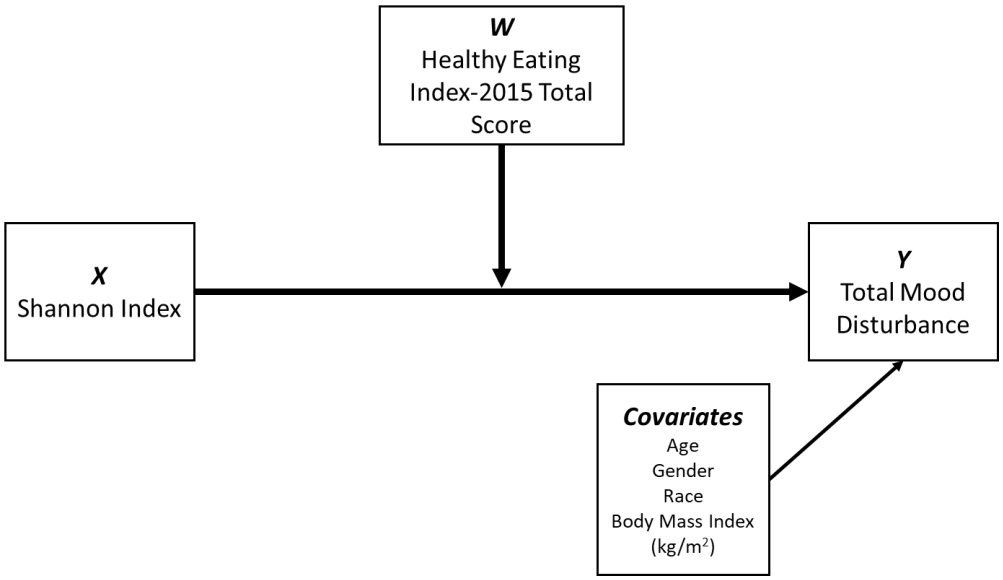

Figure 4.1. Simple moderation of intestinal alpha diversity measured by Shannon Index (X) on Total Mood Disturbance score from the Profile of Mood States questionnaire (Y) with Healthy-Eating Index total score (W) as a moderator. Age, gender, race, and body mass index (kg/m2) are included in the model as covariates. (*p*-value= 0.253)

4.4 Results

Of the 85 participants in the two cohorts, 75 had complete microbiome and POMS data and thus were included in this investigation. Table 4.1 reports demographic characteristics. Participants were mostly white (67%), 54.5 years old (±11.8), and overweight (BMI 28.5 ± 6.5 kg/m^2). No differences in demographics were observed between low MD and high MD.

Table 4.2 reports dietary intake for MD groups. Participants consumed an average of 2008.4 calories (± 560.5), with about 17% of calories from protein. There were no significant

differences in diet components between the MD groups (p>0.05 for all variables). No differences were observed in HEI component or total scores between MD groups.

Table 4.1. Demographic characteristics of adults in a cross-sectional analysis dichotomized by low and high mood disturbance scores

Variable	All (n=75)	Low Mood Disturbance (n=37)	High Mood Disturbance (n=38)	*p*-value
Age (years)*	54.5 (11.8)	56.0 (11.7)	51.6 (11.8)	0.114
Body Mass Index (kg/m^2)*	28.5 (6.5)	27.6 (6.2)	28.1 (4.1)	0.735
Body Fat (%)*	32.9 (8.2)	31.7 (7.3)	33.8 (9.1)	0.283
Total Mood Disturbance (TMD)*	87.1 (12.7)	76.7 (5.7)	97.2 (8.7)	<0.001
TMD$	86.0 (65.0-124.0)	78.0 (65.0-85.0)	96.5 (86.0-124.0)	
Sex				0.486
Female#	46 (56.1)	23 (62.2)	20 (52.6)	
Male#	36 (43.9)	14 (37.8)	18 (47.4)	
Race				0.698
Asian#	3 (3.7)	1 (2.7)	2 (5.3)	
Black#	11 (13.4)	5 (13.5)	4 (10.5)	
White#	67 (81.7)	31 (83.8)	31 (81.6)	
Other#	1 (1.2)	0 (0)	1 (2.6)	
Body Mass Index Category				0.541
Underweight#	2 (2.4)	1 (2.7)	1 (2.6)	
Healthy#	16 (19.5)	8 (21.6)	6 (15.8)	
Overweight#	39 (47.6)	20 (54.1)	17 (44.7)	
Obese#	25 (30.5)	8 (21.6)	14 (36.8)	

*Data presented as mean (SD); $data presented as median (range); #data presented as N (%)

Table 4.2. Dietary elements and Healthy Eating Index-2015 (HEI) component scores for mood disturbance groups

	All (n=75)	Low Mood Disturbance (n=37)	High Mood Disturbance (n=38)	*p*-value
Nutrients				
Calories (kcal)	2,008.4 (560.5)	1,984.49 (498.26)	2,190.14 (574.63)	0.140
Protein (g)	87.3 (28.5)	84.60 (24.73)	94.43 (32.52)	0.186
Fat (g)	84.7 (30.4)	83.19 (26.31)	92.61 (33.88)	0.228
Carbohydrate (g)	212.3 (74.8)	208.54 (75.87)	233.22 (72.09)	0.199
Sugar (g)	53.5 (50.1)	56.51 (48.77)	50.17 (55.01)	0.635
Fiber (g)	25.5 (11.0)	24.72 (11.41)	26.12 (10.38)	0.597
Healthy Eating Index (HEI) components				
Total Fruit	2.5 (1.8)	2.5 (1.9)	2.4 (1.5)	0.688
Whole Fruit	2.9 (2.0)	2.9 (2.0)	2.9 (2.0)	0.889
Total Vegetable	3.4 (1.5)	3.4 (1.6)	3.3 (1.4)	0.807
Greens and Beans	2.6 (2.3)	2.7 (2.5)	2.8 (2.2)	0.782
Whole Grains	2.2 (2.7)	1.9 (2.8)	2.6 (2.7)	0.268
Refined Grains	7.7 (3.3)	7.8 (2.7)	7.7 (3.8)	0.915
Dairy	4.8 (2.9)	5.0 (3.2)	4.6 (2.6)	0.589
Protein Foods	4.6 (0.9)	4.7 (0.7)	4.6 (0.8)	0.633
Seafood and Plant Protein	3.1 (2.2)	2.9 (2.4)	3.4 (1.9)	0.345
Fatty Acids	5.5 (3.2)	5.7 (3.2)	5.4 (3.0)	0.621
Sodium	4.2 (3.4)	4.4 (3.3)	3.9 (3.4)	0.571
Added Sugars	7.4 (2.7)	7.7 (2.2)	7.1 (3.0)	0.382
Saturated Fat	5.0 (3.4)	5.0 (3.1)	5.2 (3.7)	0.875
Total HEI Score	55.9 (12.1)	56.6 (12.3)	55.9 (12.4)	0.801

Table 4.3 presents alpha diversity measures by MD groups. Shannon and Simpson indices indicate higher alpha diversity in participants with low MD compared to high MD (p=0.004 and p=0.008, respectively).

Table 4.3. Differences in fecal microbiome alpha diversity metrics of mood disturbance groups

	All (n=75)	Low Mood Disturbance (n=37)	High Mood Disturbance (n=38)	*p*-value
Observed Species	135.2 (59.8)	145.7 (54.2)	119.5 (61.3)	0.060
Whole Tree Phylogeny	13.3 (4.9)	14.1 (4.1)	12.2 (5.7)	0.099
Shannon Index	4.95 (1.00)	5.23 (0.65)	4.55 (1.19)	**0.004**
Simpson Index	0.91 (0.10)	0.94 (0.04)	0.88 (0.14)	**0.008**

Table 4.4 reports MD group means and differences in abundance of the ten most prevalent genera among the samples. No significant differences were observed.

Table 4.4. Top 10 genera in fecal microbiome of mood disturbance groups in a cross-sectional analysis

Genus	All (n=75)	Low Mood Disturbance (n=37)	High Mood Disturbance (n=38)	*p*-value
Blautia	0.266 (0.157)	0.257 (0.140)	0.292 (0.184)	0.591
Bacteroides	0.067 (0.086)	0.060 (0.070)	0.076 (0.105)	0.825
Faecalibacterium	0.062 (0.055)	0.068 (0.048)	0.053 (0.052)	0.155
[Eubacterium] hallii group	0.028 (0.021)	0.032 (0.021)	0.025 (0.019)	0.174
Fusicatenibacter	0.030 (0.026)	0.029 (0.026)	0.030 (0.026)	0.869
Agathobacter	0.033 (0.034)	0.034 (0.027)	0.035 (0.043)	0.486
Anaerostipes	0.028 (0.042)	0.021 (0.021)	0.039 (0.058)	0.341
Ruminococcus	0.027 (0.032)	0.027 (0.027)	0.027 (0.039)	0.440
Subdoligranulum	0.023 (0.027)	0.025 (0.024)	0.017 (0.018)	0.234
Roseburia	0.022 (0.020)	0.022 (0.019)	0.019 (0.019)	0.270

Figure 4.2 displays correlations between alpha diversity metrics, dietary intake, and POMS subscales in the whole sample. Both Simpson and Shannon indices were positively correlated with the negative subscale of anger (rho= -0.303, *p*=0.011; rho= -0.265, *p*=0.027, respectively) and total MD (rho= -0.404, *p*=0.001; rho= -0.357, *p*=0.002, respectively). Simpson index was negatively associated with the subscales of depression (rho= -0.259, *p*=0.03) and confusion (rho= -0.270, *p*=0.024). Both Simpson and Shannon indices were positively associated with the two positive

subscales: vigor (rho=0.307, p=0.01; rho=0.279, p=0.019, respectively) and esteem-related affect (rho=0.302, p=0.011; rho=0.280, p=0.019, respectively). Refined grain component score was positively associated with the fatigue subscale (rho=0.428, p<0.001), tension subscale (rho=0.302, p=0.014), and confusion subscale (rho=0.253, p=0.042). The effect sizes of significant correlations were less than 0.5 and therefore reflected low to moderate effects [275].

A simple moderation analysis was conducted to investigate the moderating effect of DQ on the relationship between alpha diversity (Shannon Index) and MD in healthy adults. The overall model was not significant (F(7, 53) = 2.00, p=0.072, R^2=0.209). Shannon index was a significant predictor of MD (b= -4.39, t(53)= -2.55, p=0.014), but HEI score and the interaction (Shannon index*HEI score) were not (b=0.096, t(53)=0.66, p=0.515; b= -0.01, t(53)= -0.09, p=0.931). Although the interaction was not significant, the Johnson-Neyman results revealed a significant relationship between alpha diversity and MD (b= -4.32, t(53)= -2.01, p=0.050; b= -4.59, t(53)= -2.01, p=0.050) for individuals with an HEI score ranging from 50-71.

4.5 Discussion

This study explored the relationships among gut microbial composition, MD, and DQ in generally healthy adults from two cohorts. We observed significant differences in microbial composition between individuals with low and high MD. We also observed associations between POMS subscales and alpha diversity of the gut microbiome, as well as between DQ and POMS subscales.

Increasing evidence suggests that microbial diversity of the gut (i.e., richness) can be a potential marker of disease status [306, 307]. Specifically, reduced microbial diversity is theorized to increase risk of several diseases including functional bowel disorders and mood disorders [308, 309]. Substantiating this theory, lower alpha diversity was associated with higher depressive mood

states in our sample. Greater microbial diversity has been related to better emotional well-being, such as more positive emotions and less feelings of loneliness [310, 311], and another study by Shin et al. reported gut microbial diversity was associated with negative affect scores [312]. Our study confirms these results, with multiple negative POMS subscales (i.e., depression, anger, fatigue, and confusion) correlating with alpha diversity metrics. However, Shin et al. measured MD via the Positive and Negative Affect Schedule (PANAS) and calculated alpha diversity using the Faith's phylogenetic diversity index. Diet was also not reported by this group; our moderation analysis revealed a significant relationship between alpha diversity and MD in participants with an average HEI score, indicating the effect of diversity on mood is conditional.

The link between diet and mood has been previously investigated in a variety of contexts. One study by Wade et al. reported improvements in total MD scores from POMS following an 8-week Mediterranean dietary intervention with an extra dairy component [313]. Alternatively, Francis et al. did not observe changes in POMS subscale scores after a 3-week dietary intervention with emphasis on Mediterranean-style diet elements [314], although the length of the intervention may confound the comparison of these results. Torres et al. found a negative association between fruit consumption and the confusion subscale of the POMS in postmenopausal women following a Dietary Approaches to Stop Hypertension (DASH) diet pattern [175]. This observation was not seen in the current study, although we did see a positive association between the confusion subscale and refined grain HEI component score. Diet plays a major role in both gut microbial composition and mood-related symptoms. Maldonado-Contreras et al. observed a negative relationship between HEI total score and alpha diversity in Caribbean females, which was not present in the current study [315].

4.5.1 Limitations

Several limitations of the current study must be acknowledged. First, the collection of dietary recalls was not uniform across the studies. Instead, PP participants provided food logs where DIM participants were guided through a 24-hour dietary recall with an RD. Second, body composition techniques differed between studies, and this remains an unresolved limitation. Third, while this analysis builds on current evidence relating mood states to the gut microbiome in humans, causation could not be established. Additionally, as is consistent with secondary analyses, the current study is not powered to elucidate definitive conclusions, particularly with the moderation analysis. Lastly, our cohort is not demographically similar to the US with minimal representatives of Asian descent (3.7% of sample versus 5.4% of US population [316]). Therefore, results cannot be accurately generalized to other populations. More research is necessary to determine if manipulation of the gut microbiome can relieve MD. In this regard, several reviews have suggested that pre- and probiotics can be used in the treatment of mood disorder symptoms [223, 232, 233, 286] despite the lack of data in this area at large. Thus, this practice should be further examined.

4.5.2 Conclusions

Both diet and alpha diversity of the gut microbiome are related to mood disturbance in free-living adults. Specifically, depressive mood states have a negative relationship with alpha diversity, while vigor has a positive relationship with alpha diversity measures. However, HEI was not a moderator of the relationship between total mood disturbance and alpha diversity. With the increasing prevalence of mood disorders, further investigation is warranted to understand how diet and gut microbes may affect mood disturbance.

	Profile of Mood States Subscales								Alpha Diversity			
	Tension	Depression	Anger	Vigor	Fatigue	Confusion	Esteem Related Affect	Total Mood Disturbance	Observed Species	Whole Tree Phylogeny	Shannon Index	Simpson Index
Observed Species	-0.103	-0.185	-0.224	0.186	-0.165	-0.118	0.010	-0.211		**0.968**	**0.684**	**0.564**
Whole Tree Phylogeny	-0.089	-0.181	-0.234	0.194	-0.195	-0.162	-0.026	-0.215	**0.968**		**0.679**	**0.567**
Shannon Index	-0.095	-0.227	**-0.265**	**0.279**	-0.102	-0.218	**0.280**	**-0.357**	**0.684**	**0.679**		**0.956**
Simpson Index	-0.109	**-0.259**	**-0.303**	**0.307**	-0.145	**-0.270**	**0.302**	**-0.404**	**0.564**	**0.567**	**0.956**	
HEI Total Fruit	0.084	0.226	0.111	-0.001	0.127	0.093	0.174	0.032	0.053	0.001	0.108	0.151
HEI Whole Fruit	0.060	**0.300**	0.155	0.026	0.164	0.107	0.189	0.033	-0.001	-0.056	0.099	0.138
HEI Total Vegetable	0.143	0.097	0.007	-0.023	0.184	0.128	0.151	0.022	0.175	0.133	0.126	0.116
HEI Greens and Beans	0.062	0.072	0.034	-0.091	0.084	0.044	-0.068	0.106	-0.034	-0.074	0.022	-0.034
HEI Whole Grains	0.104	0.164	0.201	-0.185	**0.268**	0.015	0.019	0.182	-0.091	-0.121	-0.079	-0.069
HEI Refined Grains	**0.302**	0.208	0.223	-0.051	**0.428**	**0.253**	-0.011	0.238	-0.035	-0.042	-0.063	-0.061
HEI Dairy	-0.011	0.186	0.069	0.072	0.174	-0.044	0.107	-0.024	-0.086	-0.048	-0.040	-0.041
HEI Protein Foods	-0.043	0.109	0.050	-0.053	-0.108	-0.146	0.146	-0.063	-0.230	**-0.258**	-0.110	-0.121
HEI Seafood and Plant Protein	-0.029	0.154	0.102	-0.105	0.165	0.139	0.015	0.117	-0.165	-0.199	-0.119	-0.121
HEI Fatty Acids	-0.229	-0.087	-0.035	-0.017	-0.167	-0.157	0.127	-0.145	-0.178	-0.181	-0.098	-0.100
HEI Sodium	-0.092	-0.053	-0.002	-0.049	-0.139	-0.040	0.062	-0.066	-0.047	-0.051	0.026	0.062
HEI Added Sugar	0.000	0.006	-0.166	0.001	-0.107	0.041	0.086	-0.092	-0.075	-0.039	0.136	0.169
HEI Saturated Fat	-0.230	-0.059	-0.074	-0.100	-0.155	-0.133	-0.026	-0.036	-0.136	-0.182	-0.231	-0.209
HEI Total Score	-0.035	0.180	0.115	-0.054	0.091	-0.053	0.215	-0.036	-0.222	**-0.260**	-0.088	-0.038
Body Mass Index	-0.009	**-0.233**	**-0.316**	-0.139	-0.215	-0.160	-0.215	0.016	-0.115	-0.075	-0.018	0.043

Body Fat Percent	0.036	-0.154	**-0.240**	-0.132	-0.126	-0.154	-0.082	0.011	-0.022	-0.020	0.018	0.032

Figure 4.2. Correlations heatmap between POMS subscales, alpha diversity, and Healthy Eating Index (HEI) components in generally healthy adults. Shade of color indicates strength of correlation, with red representing negative relationships and blue representing positive relationships. Bold indicates significant uncorrected p-value ($p<0.05$). *Healthy Eating Index = HEI

Chapter 5: Fecal microbe diversity mediates the inverse relationship between increased lean mass and mood disturbance in young adults after 10 weeks of resistance training

5.1 Abstract

Purpose: The gut microbiome contributes to numerous physiological processes in humans and diet and exercise are known to alter both microbial composition and mood. We sought to explore the effect of a 10-week resistance training (RT) regimen with or without peanut protein supplementation (PPS) in untrained young adults on fecal microbiota and mood disturbance. Methods: Participants were randomized into PP (n=25) and control (CTL [no supplement]; n=24) groups and engaged in supervised, full-body RT twice a week. Measures included body composition, fecal microbes from 16s rRNA gene sequencing, dietary intake at baseline and following the 10-week intervention and post-intervention mood disturbance via the profile of mood states (POMS) questionnaire. Independent samples t-tests were used to determine differences between PP and CTL groups. Paired samples t-tests were used to investigate differences within groups. Results: Our sample was mostly female (69.4%), white (87.8%), normal weight (body mass index 24.6±4.2 kg/m^2), and 21±2.0 years old. Shannon index significantly increased from baseline in the whole group, yet no differences were observed between groups. Changes in *Blautia* abundance were associated with the positive POMS subscales, Vigor and self-esteem-related-affect (SERA) (rho= -0.451, p=0.04; rho= -0.487, p=0.025, respectively). Whole tree phylogeny changes were negatively correlated with SERA and Vigor (rho= -0.475, p=0.046; rho= -0.582, p=0.011, respectively) as well as change in bodyfat percentage (rho= -0.608, p=0.007). Mediation analysis results indicate change in PD Whole Tree Phylogeny significantly mediated the relationship between change in Fat Free Mass and total MD (b = 4.45, t (28)= 2.754, p=0.0102), although additional covariates of race and sex did not improve the model. Conclusions:

Mood state subscales are associated with changes in microbial taxa and body composition, and microbial diversity increased following a 10-week RT regimen. Microbial diversity may predict the relationship between changes in body composition and mood disturbance. Further research is warranted.

5.2 Introduction

The GI tract is home to the largest collection of microbes in the human body [3] and their collective genome is known as the gut microbiome. In recent years, research interest into the gut microbiome has vastly increased due to its considerable role in human health [317], including cytokine production [318], metabolism [87, 142], inflammation [13], response to stress [319], and adaptations to exercise [320]. Greater microbial diversity is beneficial to host health and can be affected by lifestyle factors, including diet and exercise [135, 321, 322]. Certain commensal bacteria can produce neurotransmitters, such as serotonin [219, 221]. Moreover, the gut microbiome affects signaling in the central nervous system via the gut-brain axis [323].

Consumption of whey or pea protein extract has been shown to increase commensal bacteria in the gut environment [324, 325]. Protein supplements are often consumed by athletes [326] due to their efficacy in increasing strength and lean body mass [327]. Cronin et al. reported altered diversity after 8-week supplementation with whey protein. Findings from this study also showed a trend towards increased microbial diversity following a combined aerobic and RT intervention in sedentary adults [328]. Similarly, in a human cross-sectional study, professional rugby athletes consuming higher protein diets displayed greater gut microbial diversity compared to lean sedentary controls [43]. Alternatively, the gut microbiome may impact physical performance [322]. Estaki et al. reported correlations between microbial diversity and cardiorespiratory fitness [329]. Nay and colleagues demonstrated reduced muscle contractile

function in mice fed antibiotics [330].Several rodent studies have indicated that exercise alters gut microbial composition and function [52, 142, 331-333]. Exercise has been shown to alter microbial diversity in humans as well, although this research is limited [42, 53].

Another suggested benefit of exercise is the improvement in emotional well-being and mood [334]. Mood disturbance is defined by feelings of distress or sadness, and symptoms of anxiety and depression [58]. Accordingly, mood-related symptoms are common in chronic diseases such as cancer, [159], diabetes [162], and HIV [160]. Participating in a 10-week RT program reduced depressive symptoms in individuals at high risk for type 2 diabetes [335], and a 6-week combined aerobic and RT program improved mood disturbance in patients with HIV [336]. There are many proposed mechanisms, including increased serotonin production [68], improved activity of central nervous system [337], and increased neurogenesis [338].

Our previous investigation in an older adult population found that peanut protein (PP) supplementation did not alter gut microbiome diversity, however, it did increase genera with metabolically beneficial gene pathways [339]. Because of the similar physiological effects of the gut microbiome and exercise, the purpose of this study was to explore the effect of PP during a 10-week RT regimen in untrained young adults on gut microbial diversity and mood disturbance.

5.3 Methods

For this secondary analysis, we assessed diet, mood, and fecal microbiome composition in young female adults participating in a 10-week RT intervention. Auburn University's Institutional Review Board (IRB) reviewed and approved the study protocol prior to any data collection (Protocol # 19-249 MR 1907) and this study was pre-registered as a clinical trial (NCT04707963; registered 13 January 2021). Detailed methods have been previously described [340].

5.3.1 Participants

Participants were recruited from Auburn University's campus and surrounding area using emails, flyers, and word of mouth. Eligibility criteria included 1) 18-30 years old; 2) body mass index (BMI) <35 kg/m2; 3) not actively participating in RT more than one time per week for six months prior; 4) no known peanut allergy; 5) free of metal implants that may interfere with x-ray procedures; 6) no medically necessary radiation exposure for six months prior; 7) free of obvious cardiovascular or metabolic disease; 8) free of conditions contraindicating participation in exercise program or donation of muscle biopsy (i.e., blood thinners or blood clotting disorder); could not be pregnant or trying to become pregnant. Eligible participants were informed of all study procedures prior to signing informed consent. Only female participants and data are included in the present analysis.

5.3.2 Study Design

For baseline (T1-1) procedures, participants reported to the Kinesiology Building at Auburn University for testing battery. First, a rapid pregnancy test was taken by female participants. Next, height and weight were measured using laboratory scales, with values rounded to the nearest 0.1 kg and 0.5 cm. Whole-body dual energy x-ray absorptiometry (DEXA) was utilized to determine body composition. Participants were randomized to either the peanut protein supplementation (PPS) or control (CTL) group. Each participant was provided with a three-day food log and stool specimen collection kit to complete and bring with them at the next appointment (T1-2). This next appointment occurred three days after T1-1 and included the first round of strength testing on bilateral leg press, barbell bench press, and hex-bar deadlift. Study staff trained and supervised participants on proper form throughout each training session. After completing the baseline strength assessment, the PP group consumed their first supplement shake and CTL did not consume a nutritional shake but were provided water. The following ten weeks after T1-2

consisted of twice weekly training sessions for a total of 20 sessions. Each training session included 1) a general warm-up of 25 jumping jacks and 10 bodyweight squats; 2) specific warm-up of 1 set of 10 repetitions (reps) at 50% working weight, 1 set of 5 reps at 75% working weight, and 1 set of 3 reps at 90% working weight and 3) either 4 sets of 10 reps (high volume) or 5 sets of 6 reps (high load) at working weight. For the first 4 weeks, weekly loads increased by ~5% for high volume day and ~9% for the high load day. Week 5 was reduced load training, consisting of 50% intensity for both high volume and high load training days. Loads were pre-programmed for all participants; however, rating of perceived exhaustion (RPE) was collected after each set to ensure appropriate loads were implemented throughout. During the last training session, strength testing was conducted on the same exercises previously discussed. Participants were provided another set of three-day food logs and stool collection kits for post-intervention testing (T-3), which was conducted 72-hours after strength testing. The battery of tests conducted at baseline are repeated for T-3, including pregnancy test, height and weight, DEXA, and submission of food-logs and stool sample.

5.3.3 Peanut Protein Supplement

Participants in the PP group were provided PBFit (BetterBody Foods, Lindon, UT, USA) to consume on non-workout days and study staff would prepare the supplement and supervise the consumption on workout days. Participants were instructed to not consume the supplement as a meal replacement. The PP supplement prepared by study staff was mixed with ~16 ounces of tap water with 75 grams of the PBFit powder to provide 30 g/day protein, >9.2g/day essential amino acids, and ~315 kcal. Amino acid content per daily serving has been described previously [340].

5.3.4 Dietary Analysis

Three-day food logs were completed by participants and included nutritional intakes for one weekend day and two weekdays leading up to testing day. Participants were asked to maintain normal dietary habits throughout the duration of the study protocol, aside from the supplement for individuals in the PP group. Data from the food logs were entered into the Nutrition Data System for Research (NDSR; NDSR 2014; University of Minnesota) [341]. Calories, micro- and macronutrients, and food groups were averaged from the three days to obtain a mean intake at each timepoint.

5.3.5 Fecal Sample Processing

Participants were given a commode specimen collection kit with a sterile collection tube and instructed to store sample in freezer until submission at next appointment. Upon receipt, samples were stored at -80°C prior to batch processing. Fecal microbial DNA was isolated using Zymo Research kits (Irvine, CA, USA). DNA samples were prepared, and polymerase chain reaction (PCR) amplified the 250 base pair variable region 4 of 16s rRNA. Sequencing of the PCR library was conducted using the Illumina Miseq (San Diego, CA, USA). The Quantitative Insight into Microbial Ecology (QIIME) suite, version 1.7 using DADA2 was used to generate amplicon sequence variants (ASVs) with a similarity threshold of 97% [301, 302]. Taxonomic assignments were defined using the SILVA database [342].

5.3.6 Mood Disturbance

Mood disturbance (MD) was assessed using the abbreviated Profile of Mood States (POMS) rating scale. Individuals are asked to rate their mood using a 5-point Likert scale ranging from “not at all” to “extremely” based on 40 different mood states. This questionnaire measures seven mood dimensions: 1) tension/anxiety; 2) anger/hostility; 3) vigor/activity; 4) fatigue/inertia; 5) depression/dejection; 6) confusion/bewilderment; 7) self-esteem-related affect (SERA). The

positive subscales are vigor and SERA, while the remaining five subscales are considered negative. To calculate total MD, the total negative subscales (tension + anger + fatigue + depression + confusion) are subtracted from the total positive subscales (vigor + esteem-related affect), with a constant (100) added to eliminate negative total MD scores. A higher score indicated greater MD.

5.3.7 Statistical Analysis

All statistical analysis was conducted using SPSS Version 25.0 (IBM Corp, Armonk, NY, USA) and statistical significance was set at $p<0.05$. Data are presented as mean ± standard deviation throughout, unless otherwise indicated. Chi-square tests analyzed differences in categorical variables between groups. Spearman correlations were conducted in the whole group to explore relationships between changes in alpha diversity, changes in body composition, and POMS subscales. Independent samples t-test explored differences in continuous variables between intervention groups at baseline and follow-up. Paired samples t-tests assessed differences in diet, microbiome, body composition, and MD variables within intervention groups. Spearman correlations were performed within intervention groups to explore relationships between POMS subscales and the 25 most abundant genera detected in samples. Alpha diversity was measured using Observed Species, PD Whole Tree Phylogeny, Shannon index, and Simpson index. Beta-diversity was measured using Bray Curtis and Unweighted and Weighted Unifrac metrics. Kruskal-Wallis one-way analysis of variance (ANOVA) tests with false discovery rate (FDR) corrections were performed to compare relative abundance of all ASVs at all phylogenic levels between intervention groups at both time points.

5.3.7.1 Mediation Analysis

To explore the mediating effect of change in alpha diversity on the relationship between FFM changes and MD, a simple mediation analysis was conducted in the whole sample using

PROCESS [274]. The outcome variable was POMS total MD (*Y*), the predictor variable was change in FFM (*X*), and the mediator was alpha diversity, measured using PD Whole Tree Phylogeny (*M*). Sex and race were included as covariates due to confounding differences in these variables. Mediation analysis was conducted to explore indirect effects, with significant effects supported by the absence of zero within the confidence intervals.

5.4 Results

A total of 109 individuals expressed interest in participating and 56 individuals met eligibility criteria for enrollment in the study. Forty-nine participants completed the 10-week RT intervention. Figure 5.1 details participant retention and dropout. Table 5.1 reports demographic characteristics of the study sample and within the supplementation groups. Participants were mostly white (87.8%), female (69.4%), 21±2.0 years of age and normal weight (BMI 24.6 ± 4.2 kg/m^2). There were no significant differences in demographics between PP and CTL groups.

Average daily intakes of macronutrients and dietary components are reported in Table 5.2. No differences in diet variables between groups were observed at baseline. From baseline to follow-up, participants significantly decreased fat consumption (from 73.7 ± 29.8 to 64.7 ± 24.6; p=0.044). At follow-up the PP group reportedly consumed more protein (91.1 ± 28.2 vs. 67.8 ± 24.7; p=0.004) and fiber (23.8 ± 5.4 vs. 14.4 ± 7.7; p<0.001) compared to the CTL group.

Table 5.3 presents body compositional changes with the intervention and differences between groups. Whole group changes included increased weight (74.1 ± 14.2 to 75.0 ± 18.1 kg; p=0.024), increased FFM (47.2 ± 9.6 to 48.6 ± 10.1 kg; p<0.001), decreased body fat (BF) % (31.3 ± 8.0 to 30.7 ± 7.8; p=0.012), and increased BMI (24.6 ± 4.2 to 24.9 ± 4.4 kg/m^2). Both groups significantly increased FFM (PP: 46.4 ± 9.5 to 47.7 ± 9.1, p<0.001; CTL: 48.1 ± 9.8 to 49.4 ± 11.1;

p=0.002), while only the PP group decreased BF% from baseline to follow-up (31.1 ± 8.0 to 30.2 ± 7.8, p=0.014).

Table 5.4 reports alpha diversity metric differences between intervention groups. Shannon Index significantly increased from baseline to follow-up in the whole group (4.97 ± 0.77 to 5.09 ± 0.71 p=0.040). No differences were observed between intervention groups.

MD subscales are presented in Table 5.5. Participants in the PP group reported greater feelings of Vigor compared to the CTL group (9.2 ± 4.2 vs. 6.6 ± 3.6; p=0.023). Beta diversity, as measured by Unweighted Unifrac, was significantly different (p=0.028) between intervention groups, however, after FDR corrections, no differences remained between taxa.

Within the PP group, the genus *Subdoligranulum* positively associated with the SERA POMS subscale (rho= 0.458; p=0.037) and *Romboutsia* associated with the Vigor subscale (rho=0.465, p=0.034). The Fatigue subscale was negatively correlated with *Ruminococcus* 1 and *Ruminococcus* 2 (rho= -0.465, p=0.034; rho= -0.504, p=0.02, respectively). *Anaerostipes* correlated with Anger subscale (rho=0.540, p=0.011) and total MD score (rho=0.510, p=0.018). Changes in observed species were associated with changes in FFM (rho=0.470, p=0.049). PD Whole tree phylogeny changes were negatively correlated with change in %BF (rho= -0.608, p=0.007), as well as SERA and Vigor (rho= -0.475, p=0.046; rho= -0.582, p=0.011) and positively associated with the Depression subscale (rho=0.496, p=0.036). Changes in *Blautia* abundance were associated with both positive POMS subscales, Vigor and SERA (rho= -0.451, p=0.04; rho= -0.487, p=0.025, respectively). Changes in *Subdoligranulum* abundance was positively correlated with Vigor subscale and changes in FFM (rho=0.530, p=0.014; rho=0.469, p=0.032, respectively).

Within the CTL group, the Anger subscale negatively correlated with *Roseburia* and *[Eubacterium] hallii group* (rho= -0.713, p=0.004; rho= -0.540, p=0.046, respectively), and

positively correlated with *Bifidobacterium* and *Romboutsia* (rho= 0.540, p=0.046; rho= 0.713, p=0.004, respectively). The Depression subscale was negatively associated with *Roseburia* and *Lachnospiraceae_unclassified* (rho= -0.573, p=0.032; rho= -0.539, p=0.047, respectively). The Confusion subscale was positively correlated with *Coprococcus* 3 and *Anaerostipes* (rho=0.537, p-value=0.047; rho=0.578, p-value=0.031, respectively). The Fatigue subscale was positively associated with changes in both PD Whole Tree Phylogeny and Simpson (rho=0.594, p=0.019; rho=0.678, p=0.006). Changes in *Subdoligranulum* abundance was negatively correlated with the Anger, Fatigue, and Depression subscales (rho=0. -540, p=0.046; rho= -0.734, p=0.003; rho= -0.551, p=0.041, respectively). In the CTL group, no correlations were observed between changes in top 25 genera and body composition.

Because of the relationships between POMS subscales and body composition changes, and POMS subscales and alpha diversity, we explored the mediating effects of the gut microbiome on the relationship between body composition changes and MD. The outcome variable was POMS total MD (*Y*), the predictor variable was change in FFM (*X*), and the mediator was PD Whole Tree Phylogeny (i.e., alpha diversity) (*M*). Results from the simple mediation analysis indicate change in PD Whole Tree Phylogeny significantly mediated the relationship between change in FFM and total MD (b = 4.45, t(28)= 2.754, p=0.0102). Figure 5.2 reports model design with unstandardized regression analysis. With addition of race and sex as covariates, the model was not improved (p>0.05 for both). The indirect effect of FFM on total MD was not significant (indirect effects= -0.027, SE = 0.708, 95% CI [-1.45, 1.54]). Therefore, gut microbial changes mediate the relationship between FFM changes and MD.

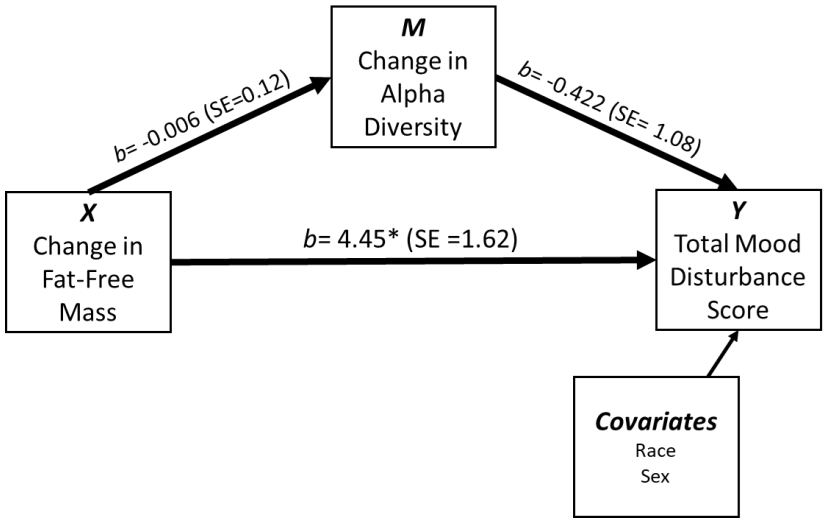

Figure 5.2. Simple mediation of Fat-Free Mass changes (X) on Total Mood Disturbance Score (Profile of Mood States) with Change in alpha diversity (as measured by PD Whole Tree Phylogeny) as a mediator. Race and sex are covariates in this model. Unstandardized regression coefficients are reported with standard error values; *p-value <0.05

5.5 Discussion

This study explored the effects of PP on diet, gut microbiota, and mood disturbance after 10 weeks of RT. We observed an increase in alpha diversity as measured by Shannon Index in the whole group and no differences between intervention groups. Additionally, several genera were related to POMS subscales. These results indicate RT can improve microbial diversity in younger untrained adults, however, only one measure of alpha diversity was improved. After a 6-week endurance-based training regimen in obese and lean participants, Allen et al. found beta-diversity changes were dependent on BMI and any changes in the microbiota were reversed after terminating training [42]. Similarly, Cronin et al. reported that moderate-intensity mixed aerobic and RT for 8 weeks resulted in modest increases in microbial diversity although this only trended towards significance [53]. However, the training protocols in these studies differed from ours in the incorporation of aerobic exercise, which could explain differences in our results. Notably, aerobic

exercise can modify microbial diversity and abundance of certain genera, although investigations into the effects of resistance or strength training are limited.

In our study, *Roseburia* was negatively associated with the Anger and Depression subscales in the CTL group. In anorexia nervosa patients, decreased fecal *Roseburia* has been reported compared to healthy controls [343]. *Roseburia* is known to produce butyrate, a short chain fatty acid (SCFA) that sustains colonocytes and is beneficial for inflammation and intestinal barrier function [344]. Butyrate has also shown anti-depressive properties in mice by reducing behaviors related to cognitive and social impairments and low energy [225, 345]. In the context of PA, individuals with greater aerobic fitness produce more butyrate [329]. While SCFAs were not measured in the current study, two negative subscales (Anger and Depression) had an inverse relationship with the genus *Roseburia* which could be due to the inferred production of SCFAs. Alternatively, obese children showed increased levels of *Roseburia* after participating in a 12-week endurance and RT program, in similar abundance to the healthy control group [346]. These results suggest physical activity can improve abundance of commensal bacteria, which may lead to mood-related improvements via the GBA.

Anaerostipes is another genus known to produce butyrate [347]; however, we observed a positive relationship between this genus and Anger and total MD in the PP group, while the CTL group showed a positive relationship between *Anaerostipes* and Confusion. These results differ from the butyrate-producing *Roseburia*, and other previous evidence indicating *Anaerostipes* improves gut barrier integrity and has anti-inflammatory properties [348]. Verheggen et al. found increased *Anaerostipes* following 8 weeks of aerobic training in obese individuals, although no changes were seen in alpha diversity metrics, similar to the results found herein [349].

The genus *Romboutsia* was positively associated with the Vigor subscale in the PP group, but positively associated with Anger and Fatigue subscale in the CTL group. Species within *Romboutsia* genus are able to synthesize some B vitamins, metabolize carbohydrates, and ferment amino acids [350], supporting its potentially beneficial role in human health. Alternatively, *Romboutsia* is suggested to inhibit enzymatic steps along the serotonin production pathway [351], which plays an important role in mood [352]. On the other hand, a single exercise bout in endurance athletes showed an increase in abundance of *Romboutsia* [353], while decreased *Romboutsia* was observed following exercise and vitamin C supplementation in a rat model [354]. These differences may be due to lack of precision; thus, species-level genomic analysis may reveal more insight into the impact of the *Romboutsia* genus on human health.

Consumer trends indicate plant-based protein is increasing in popularity [355], but are unproven in comparison to the benefits of whey protein [356-359]. PP is equivalent to meat and eggs for promoting health and growth in humans and equally as digestible [360, 361], and PP has the additional benefits of fiber and phytonutrients [362]. Because study protocol advised the CTL group from changing diet, the participants in the PP group consumed more fiber and protein. Dietary fiber can improve microbial composition by supplying energy to commensal bacteria [138, 139], while the effects of protein on microbiota display mixed results [41, 363]. Interestingly, the participants in the PP group experienced more feelings of vigor compared to CTL. A study showed mood improvements after including a fiber-containing cereal bar at breakfast compared to no breakfast meal [364]. The higher fiber content in the PP shake could help explain the higher vigor levels within our sample.

5.5.1 Limitations

While this study provides initial insight into PP during RT and the health benefits associated, it is not without limitations. First, there is lack of diversity with participants given that 69.4% were female and 87.8% were white. More females than males were purposefully recruited for this training study due to the substantial lack of investigation into female populations in the sports and exercise field [365]. Subsequent exploration should include a more diverse sample to extrapolate findings to the general population. Secondly, our study design prevented us from exploring the differences between whey (or another animal-based protein) and peanut protein. Future research should compare the effects of PP to other well-studied protein sources to inform recommendations on plant-based protein supplements. Moreover, collection of fecal samples occurred at various times during the day and circadian shifts in the microbiome could account for minimal differences observed [366]. Additionally, participants were responsible for keeping food logs, and human error could occur with self-reported data. Lastly, this secondary analysis is not adequately powered to establish conclusions from our current results. Further investigation is warranted to explore the effects of PP on mood disturbance and gut microbiota in adequately powered ssmples.

5.5.2 Conclusions

PA has long been suggested as beneficial to mood and affect, in part due to increased production of serotonin and other neurotransmitters [367]. Specifically, RT has been shown to reduce anxiety or depression symptoms, improve self-esteem-related affect, and alleviate fatigue [368]. The gut microbiome is a novel target that can improve mood and mental health [286]. Because of this interconnected relationship, improving composition of the microbiome and increasing PA are mechanisms by which mood can be improved with compounding health benefits

[369, 370]. Herein, we observed changes in microbial taxa related to body compositional changes and mood state subscales. We also found increased microbial diversity following a 10-week RT program, and microbial diversity may have mediated the relationship between mood disturbance and body composition changes. These results support previous research indicating changes in gut microbial composition following exercise, and the relationship between the gut microbiome and mood disturbance. Further research is necessary to explore gut microbial responses to resistance training and their compounding effects on mood.

CONSORT 2010 Flow Diagram

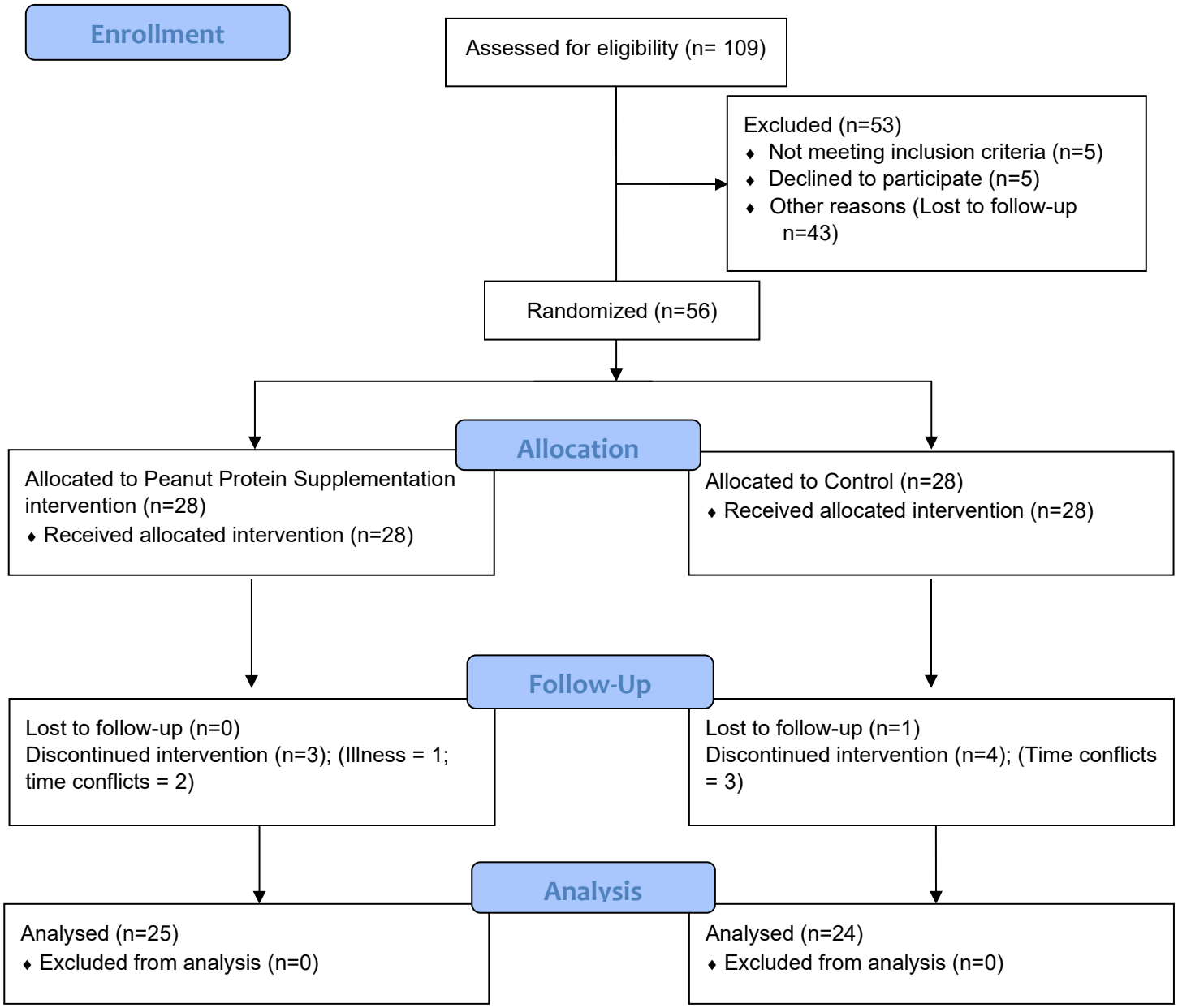

Figure 5.1. Consolidated Standards or Reporting Trials (CONSORT) Flow Diagram.

Table 5.1. Demographic characteristics of young adults participating in a 10-week resistance training study by supplementation intervention groups

	All (n=49)	Peanut (n=25)	Control (n=24)	p-value
Age*	21.1 (2.0)	21.4 (2.2)	20.8 (1.7)	0.358
Baseline Body Mass Index*	24.6 (4.2)	23.8 (3.7)	25.4 (4.7)	0.197
Sex#				0.686
Female	34 (69.4)	18 (72)	16 (66.7)	
Male	15 (30.6)	7 (28)	8 (33.3)	
Hispanic#				0.368
Cuban	1 (2)	1 (4)	0 (0)	
Another Hispanic, Latino, or Spanish Origin	1 (2)	1 (4)	0 (0)	
No Hispanic, Latino, or Spanish Origin	47 (95.9)	23 (92)	24 (100)	
Race#				0.572
Asian	1 (2)	1 (4)	0 (0)	
Black or African American	4 (8.2)	2 (8)	2 (8.3)	
White	43 (87.8)	21 (84)	22 (91.7)	
More than one race	1 (2)	1 (4)	0 (0)	
Education#				0.210
HS Grad	3 (6.1)	0 (0)	3 (12.5)	
Some College	35 (71.4)	19 (76)	16 (66.7)	
AA or AS	1 (2)	5 (20)	1 (4.2)	
Bachelors	7 (14.3)	1 (4)	2 (8.3)	
Masters	3 (6.1)	0 (0)	2 (8.3)	
Baseline Body Mass Index Category#				0.536
Underweight	2 (4.1)	2 (8)	0 (0)	
Normal	32 (65.3)	16 (64)	16 (66.7)	
Overweight	10 (20.4)	5 (20)	5 (20.8)	
Obese	5 (10.2)	2 (8)	3 (12.5)	
Marital Status#				0.966
Single	45 (91.8)	23 (92)	22 (91.7)	
Married or Domestic Partnership	4 (8.2)	2 (8)	2 (8.3)	

*Presented as mean (SD); #presented as N (%)

Table 5.2. Dietary components between supplementation intervention groups of young adults participating in a 10-week resistance training study

	p-value*	All (n=49)	p-value#	Peanut (n=25)	p-value#	Control (n=24)	p-value#
Calories (kcal)			0.285		0.640		0.255
PRE	0.187	1,640.0 (611.7)		1,753.6 (665.9)		1,521.7 (538.1)	
POST	0.063	1,547.5 (545.7)		1,689.0 (655.1)		1,400.2 (359.4)	
Protein (g)			0.385		0.120		0.825
PRE	0.318	74.8 (34.4)		79.7 (35.5)		69.8 (33.2)	
POST	**0.004**	79.7 (28.8)		91.1 (28.2)		67.8 (24.7)	
Carbohydrate (g)			0.545		0.956		0.327
PRE	0.454	170.0 (84.4)		178.9 (88.9)		160.7 (80.2)	
POST	0.102	163.7 (62.5)		178.0 (74.6)		148.7 (43.3)	
Fat (g)			**0.044**		0.076		0.300
PRE	0.134	73.7 (29.8)		79.9 (28.3)		67.1 (30.6)	
POST	0.230	64.7 (24.6)		68.8 (29.7)		60.3 (17.5)	
Fiber (g)			**0.000**		**0.000**		0.309
PRE	0.257	13.5 (6.3)		14.5 (6.5)		12.5 (6.1)	
POST	**0.000**	19.1 (8.1)		23.8 (5.4)		14.4 (7.7)	
Sugar (g)			0.215		0.803		0.129
PRE	0.810	58.0 (44.5)		56.5 (39.7)		59.6 (49.8)	
POST	0.309	51.5 (31.5)		56.1 (38.0)		46.8 (23.1)	

*Between-group p-value; #within-group p-value; bold font indicates statistical significance

Table 5.3. Body composition metrics and differences between supplementation intervention groups of young adults participating in a 10-week resistance training study

	p-value*	All (n=49)	p-value#	Peanut (n=25)	p-value#	Control (n=24)	p-value#
Weight			**0.024**		0.122		0.108
PRE	0.370	74.1 (17.2)		71.9 (14.2)		76.4 (19.9)	
POST	0.374	75.0 (18.1)		72.7 (13.3)		77.4 (22.1)	
Fat Mass			0.615		0.236		0.742
PRE	0.451	23.8 (10.2)		22.7 (8.5)		24.9 (11.8)	
POST	0.359	23.7 (10.5)		22.3 (7.8)		25.1 (12.7)	
Fat Free Mass			**0.000**		**0.000**		**0.002**
PRE	0.549	47.2 (9.6)		46.4 (9.5)		48.1 (9.8)	
POST	0.549	48.6 (10.1)		47.7 (9.1)		49.4 (11.1)	
Body Fat Percentage			**0.012**		**0.014**		0.264
PRE	0.823	31.3 (8.0)		31.1 (8.0)		31.6 (8.2)	
POST	0.687	30.7 (7.8)		30.2 (7.8)		31.2 (8.0)	
Body Mass Index			**0.029**		0.103		0.155
PRE	0.197	24.6 (4.2)		23.8 (3.7)		25.4 (4.7)	
POST	0.219	24.9 (4.4)		24.1 (3.3)		25.7 (5.2)	

*Between-group p-value; #within-group p-value; bold font indicates statistical significance

Table 5.4. Alpha diversity measures between intervention groups of young adults in a 10-week resistance training study

	p-value*	All (n=49)	p-value[#]	Peanut (n=25)	p-value[#]	Control (n=24)	p-value[#]
Observed Species			0.688		0.355		0.439
PRE	0.108	122.0 (34.2)		111.0 (32.0)		131.9 (40.7)	
POST	0.302	120.5 (37.2)		116.6 (37.4)		128.6 (30.0)	
Whole Tree Phylogeny			0.594		0.224		0.233
PRE	0.057	9.95 (2.55)		9.12 (2.29)		10.96 (2.55)	
POST	0.294	10.08 (2.50)		9.60 (2.75)		10.67 (2.09)	
Shannon Index			**0.040**		0.062		0.377
PRE	0.263	4.97 (0.77)		4.85 (0.87)		5.13 (0.61)	
POST	0.432	5.09 (0.71)		5.01 (0.83)		5.20 (0.54)	
Simpson Index			0.165		0.262		0.438
PRE	0.376	0.93 (0.06)		0.92 (0.08)		0.94 (0.03)	
POST	0.439	0.93 (0.07)		0.93 (0.09)		0.94 (0.03)	

*Between-group p-value; [#]within-group p-value; bold font indicates statistical significance

Table 5.5. Mood disturbance after a 10-week resistance training study between supplementation intervention groups

	All (n=49)	Peanut (n=25)	Control (n=24)	Between group p-value
Tension	2.8 (2.9)	3.2 (3.4)	2.5 (2.2)	0.400
Anger	0.4 (1.0)	0.4 (1.3)	0.3 (0.6)	0.712
Fatigue	3.1 (3.2)	2.9 (3.8)	3.3 (2.5)	0.690
Depression	0.5 (1.2)	0.5 (1.5)	0.5 (0.8)	0.955
Esteem-Related Affect	16.6 (3.3)	17.4 (3.3)	15.8 (3.1)	0.103
Vigor	7.9 (4.1)	9.2 (4.2)	6.6 (3.6)	**0.023**
Confusion	1.5 (2.1)	1.7 (2.0)	1.3 (2.1)	0.562
Total Mood Disturbance	83.8 (10.9)	82.2 (12.2)	85.5 (9.1)	0.286

Bold font indicates statistical significance

Chapter 6: Conclusion

This dissertation reports three studies which contribute evidence supporting the GBA as it relates to diet, body composition, PA, and mood. The first study explored QOL measures and fecal microbiome composition in older BCS. Microbial composition differed in obese and non-obese BCS, with several genera associating with QOL subscales. Of these genera, *Roseburia*, *Ruminococcus*, and *Dorea*, were observed in higher abundance in the non-obese BCS, and the latter two were negatively associated with QOL subscales. While Observed Species did not mediate the effect of BMI on MCS, addition of race as a covariate improved the model, indicating the relationship between the gut microbiome and mental health can be impacted by body mass and race. Further exploration is necessary to determine if these relationships are found in non-cancer survivor populations. The second study investigated the relationship between mood disturbance, diet quality, and the fecal microbiome of free-living adults. Higher mood disturbance was associated with lower microbial diversity, and DQ was associated with various mood state subscales. Furthermore, the Shannon Index of alpha diversity predicted mood disturbance scores, although the interaction between DQ and diversity was not helpful in predicting mood disturbance. The final study explored post-intervention mood disturbance with changes in gut microbiota, diet, and body composition after participating in a 10-week RT regimen. Several genera associated with mood dimensions in the intervention groups, with some of these previously identified as commensal. Following the RT regimen, the whole group showed increased alpha diversity.

These results provide evidence for the crucial relationship between the gut microbiome and mood, with influences from diet and PA habits. With physical and emotional stress a consistent feature of life, understanding how the gut microbiome influences the response to stressors is important to lessen the burden of mood disturbance. The GBA provides several avenues to

manipulate this relationship for mood improvements. Pre- and probiotics are already being investigated as alternative therapeutics for mood disorders, with very promising evidence in preclinical models. Future work should explore the composition of the gut microbiome and its impact on mood, and the effects of diet and PA on this relationship.

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