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Author Note

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Abstract

Recent work has found relationships between the gut microbiota—the community of organisms that inhabit an animal's digestive tract—and psychological health. In particular, the gut microbiota of individuals with depression shows a different genetic composition to those without depression. Thus, this study explored how rumination, a predictor of depression, and gut microbiota composition are correlated to detect possible gut microbiota alterations present before depression develops. This study also examined whether a brief mindfulness mobile application intervention, which has been shown to reduce rumination, can increase beneficial bacteria abundance and decrease pathogenic bacteria abundance. Participants were 16 first-year students. They engaged in a 4-week brief mindfulness mobile app intervention. Rumination was assessed by a self-report questionnaire, and participants' gut microbiota compositions were analyzed from fecal samples collected at pre- and post-intervention. There were significant correlations between rumination and three gut microbiota groups. However, the results were inconclusive due to the small sample size and inconsistency in past studies to determine whether the gut microbiota is beneficial or pathogenic. Additionally, there were significant differences in abundance from pre- to post-intervention in three taxa. The genus *Bifidobacterium*—a beneficial taxon—increased, and the genus *Marvinbryantia*—a pathogenic taxon—decreased in individuals after the intervention. However, another pathogenic genus of bacteria, *Alistipes,* increased in individuals after the intervention. Future studies should investigate the relationship between rumination and the gut microbiome with a bigger sample size. Additionally, a randomized controlled trial is needed to see the intervention efficacy alone on gut health.

Keywords: rumination, gut microbiota, mindfulness, mobile intervention

Rumination and the Gut Microbiome: Effects of a Brief Mindfulness Intervention

There are approximately 100 trillion microorganisms that inhabit the digestive tract of an animal, including bacteria, viruses, fungi, and protozoa (Valdes et al., 2018). Such a community of microorganisms is known as the gut microbiota. The gut microbiome—functional genes and metabolites of the gut microbiota—can impact host's physiology (Greenhalgh et al., 2016). For example, they can benefit the host by producing energy, vitamins, and other metabolites from the food the host consumes (Mohajeri et al., 2018). Furthermore, the gut microbiome can prevent pathogenic substances from entering the bloodstream by regulating the intestinal permeability, which protects the host from inflammation (Bäumler & Sperandio, 2016; Mohajeri et al., 2018). This mutual relationship between the gut microbiota and the host is called symbiosis (Matsuoka & Kanai, 2015). Consumption of probiotics and prebiotics has been shown to nurture such symbiosis by restoring and supporting the beneficial microorganisms (Azad et al., 2018; Yasmin et al., 2015). On the other hand, consuming antibiotics has been found to alter the proportion and composition of the gut microbiota and cause imbalance in the microbial community (Bäumler & Sperandio, 2016; Ianiro et al., 2016; Kennedy et al., 2017; Mohajeri et al., 2018). This imbalance in the microbial community is known as dysbiosis, and it can negatively impact host health. Past research has thus shown that physiological health is closely related to individuals' gut microbiota.

Recent studies have shown that the gut microbiota is also related to hosts' psychological health, including factors such as stress and depression (Jiang et al., 2015; Karl et al., 2018). In past studies, alteration of gut microbiota composition has been found in people with psychological disorders such as major depressive disorder (MDD) and generalized anxiety disorder (GAD) (Jiang et al., 2015; Lin et al., 2017; Liu et al., 2020; Rong et al., 2019). However, the larger association between the gut microbiome and

psychological health remains to be investigated. To explore how psychological health is related to the gut microbiome before the development of symptoms of clinical depression, my project examined the relationship between the gut microbiome and rumination. Rumination is a maladaptive style of responding to stress which predicts the subsequent onset of psychological symptoms (McLaughlin & Nolen-Hoeksema, 2011; Nolen-Hoeksema, 2000; Wisco & Nolen-Hoeksema, 2008). Furthermore, to explore whether improvement in psychological health can also improve the gut microbiome, the present study investigated how alleviation of rumination via a mindfulness intervention impacts the gut microbiome.

GAD and Gut Microbiome

Past research found that GAD has an association with the gut dysbiosis (Faravelli et al., 2012; Jiang et al., 2018). GAD is characterized by excessive and uncontrollable worry, which is chronic and persistent (Stein & Sareen, 2015). Jiang and colleagues (2018) reported that people with GAD have a different gut microbiota composition than their healthy counterparts. For example, organisms within the phylum Bacillota/Firmicutes, including the genera *Faecalibacterium, Roseburia, and Subdoligranulum* were all significantly reduced in people with GAD. *Faecalibacterium* is a beneficial bacteria group that produces butyric acid, one of the short-chain fatty acids (SCFAs), as its metabolite from dietary fiber (Liu et al., 2020). SCFAs have anti-inflammatory properties, and they can enhance the intestinal barrier integrity to protect the host from inflammation by preventing pathogenic substances from crossing the gut wall (Dalile et al., 2019). *Roseburia,* another genus found to be decreased in people with GAD, also produces SCFAs in the form of butyric acid. This suggests a possible relationship between a reduced abundance of butyric-acid producing bacteria—which leads to a decreased beneficial gut microbiota—and the pathology of GAD.

MDD and Gut Microbiome

People diagnosed with MDD also have different gut microbiota composition from their healthy counterparts (Jiang et al., 2015; Lin et al., 2017; Liu et al., 2020; Rong et al., 2019). MDD is characterized by emotional symptoms, such as depressed mood and hopelessness, and physical symptoms, such as fatigue and pain (Trivedi, 2006). At the phylum level, an increased abundance of Bacteroidetes, Proteobacteria, and Actinobacteria, as well as a decreased abundance of Firmicutes were observed in people with MDD (Jiang et al., 2015). Supporting this, Liu and colleagues (2020) found that people with MDD also had a lower abundance of Firmicutes. However, other studies found a greater abundance in Firmicutes and fewer Bacteroidetes in people with depression, inconsistent with the results of the aforementioned studies (Lin et al., 2017; Rong et al., 2019). Although past studies have inconsistencies in results, these studies collectively suggest that the gut microbiome is altered at the phylum level in people with MDD.

At the genus level, a more specific taxonomic rank than phylum, studies have also found differences in the gut microbiome between people with MDD and their healthy counterparts. Jiang and colleagues (2015) found overrepresented *Alistipes*—one genus represented in the gut microbiome—in people with MDD (Jiang et al., 2015). This is consistent with the results of Naseribafrouei and colleagues' study (2014), which investigated the correlation between the human gut microbiome and MDD. The genus *Alistipes* has been shown to decrease serotonin availability by breaking down serotonin's precursor, tryptophan. Decreased serotonin availability can be detrimental because it contributes to increased depressed mood (Cowen & Browning, 2015; Parker et al., 2020). This suggests that *Alistipes* is a pathogenic bacteria genus, and its activity is perhaps linked with depression (Foster et al., 2013; Naseribafrouei et al., 2014; Parker et al., 2020).

People with MDD had a reduced abundance of the beneficial genus group *Facalibacterium* (Jiang et al., 2015). The reduction in *Faecalibacterium* was more

pronounced in people with more severe depressive symptoms (Jiang et al., 2015). Similarly, the reduction of *Faecalibacterium* was seen not only in MDD, but also in GAD (Jiang et al., 2015) This suggests a sufficient abundance of this gut microbiota genus may have a protective effect on mental health (Jiang et al., 2015; Liu et al., 2020). A similar trend was also observed by Liu and colleagues (2020). Supporting the association between the decreased amount of the SCFA-producing *Faecalibacterium* in people with MDD, the concentration of SCFAs was lower in patients with depression than their healthy counterparts (Silva et al., 2020). These results suggest that people with MDD have increased pathogenic gut microbiota and decreased beneficial gut microbiota. Further research should be conducted to investigate how the abundance of pathogenic and beneficial gut microbiota are related to psychological health to better understand the holistic picture of the gut microbiota and psychological associations.

Gut Brain Axis

One mechanism through which gut microbiota composition alteration occurs with MDD and GAD may be through the communication link called the gut-brain axis (GBA). The GBA is a complex, bidirectional connection between the gastrointestinal (GI) tract and the central nervous system (CNS). This link encompasses various routes, such as the immune pathway, the autonomic nervous system (ANS), and the endocrine pathway (Cryan et al., 2019). For example, when the host is exposed to stress, the hypothalamic-pituitary-adrenal (HPA) axis, a part of the endocrine pathway, is activated (Cryan et al., 2019; Misiak et al., 2020). Although the short-term activation of the HPA axis in response to stress exposure is essential for adapting to the environment and restoring homeostasis, repeated exposure to stress can lead to overactivation of the HPA axis (Misiak et al., 2020; Pariante & Lightman, 2008). Such HPA axis abnormality has been associated with psychological disorders like MDD (Iob et al., 2020). Furthermore, research has shown that the overactivation of the HPA

axis is also associated with the gut microbiome (Misiak et al., 2020). A possible mechanism of how the HPA axis interacts with the gut microbiome is that the HPA axis activation increases the gut permeability, making the host susceptible to inflammation (Misiak et al., 2020; Vanuytsel et al., 2014). As the GBA is bidirectional, the gut dysbiosis may also contribute to HPA axis overactivation by influencing the release of proinflammatory cytokines (Misiak et al., 2020; Molina-Torres et al., 2019). Therefore, the crosstalk between the gut microbiome and the HPA axis may accelerate the progression of psychological symptoms.

Gastrointestinal Tract Disorders and Mental Health

Since the relationship between psychological factors and the gut microbiome is bidirectional, studies also show that having a disorder of the gastrointestinal (GI) tract, such as irritable bowel syndrome (IBS) or inflammatory bowel disease (IBD), is associated with some psychological disorders (Crisan & Dumitrascu., 2014). Firstly, up to 90% of patients with IBS also develop MDD in their lifetime (Friedrich et al., 2010). IBS involves chronic and relapsing abdominal discomfort and pain that affects the large intestine (Distrutti et al., 2016). One mechanism involved in the pathology of IBS is increased intestinal permeability and gut dysbiosis, suggesting that the gut microbiome may play a role in IBS symptomatology. For example, people with IBS comorbid with depression and anxiety have shown gut microbiota alteration such as higher *Prevotella* and lower *Lachnospiraceae* (Simpson et al., 2020). The altered intestinal motility due to IBS can also interact with the CNS, which may also play a role in impacting psychological health (Banerjee et al., 2017). These studies suggest that the intestinal environment may influence psychological health through GBA.

Another gastrointestinal tract disorder associated with psychological disorders is IBD. IBD includes both Crohn’s disease (CD) and ulcerative colitis (UC), and it involves chronic

inflammation in the gastrointestinal tract. A review by Graff and colleagues (2009) revealed that the life prevalence of psychological disorders (i.e., depression and anxiety) for IBD patients is 65%, and this suggests an association between psychological disorders and IBD. Multiple studies have found an imbalance of the gut microbiome in people with IBD; namely, there is decreased Firmicutes as well as increased Proteobacteria compared to healthy counterparts (Frank et al., 2007; 2015; Tong et al., 2013). Butyrate-producing bacteria such as *Faecalibacterium* were also decreased in IBD patients and may have negative impact on the pathology of IBD (Fornelos et al., 2020). Such butyrate-producing bacteria were also reduced in people with MDD and GAD, suggesting its importance in the pathology of both psychological disorders and GI tract disorders. These findings further support the relationship between gut dysbiosis and psychological disorders.

Rumination

To investigate the association between the gut microbiome and psychological health, this study first explored how rumination is associated with the gut microbiome. Rumination is a transdiagnostic risk factor that predicts the subsequent onset of psychological symptoms including anxiety and depressed mood (McLaughlin & Nolen-Hoeksema, 2011; Nolen-Hoeksema, 2000). In response to psychological stress, people often use a ruminative response style—dwelling in a negative thought loop repetitively and passively focusing on negative thoughts and stressful experiences without actively taking an action to solve the problems (Nolen-Hoeksema et al., 2008). This response style can lead to further emotional distress and eventually leads to the onset of psychological disorders (Nolen-Hoeksema et al., 2008). Therefore, rumination is a mediating factor between psychological stress and the onset of psychological symptoms (Jose & Brown, 2008).

When people ruminate in response to stressful events, they may also experience prolonged activation of the GBA, which may impact the gut microbiome. Therefore, people

with higher rumination may already be at risk of the gut microbiome alteration, even before the onset of psychological symptoms (Brosschot et al., 2006; Zoccola & Dickerson, 2012). For this reason, it is possible that the gut microbiota composition of people with high rumination may be similar to that of people with MDD and GAD. Thus, I proposed that increased rumination would be associated with increased proinflammatory/pathogenic gut microbiota and decreased anti-inflammatory/beneficial gut microbiota. Investigating whether the gut microbiome alteration occurs in those with high rumination prior to the development of psychological disorders is crucial because finding the gut microbiota composition alteration in individual with higher rumination can lead to advocating for prevention efforts for both gut and psychological health.

Mindfulness

Given that rumination is a predictor of psychological disorders, it is critical to intervene to its maladaptive response style to reduce the risk of the onset of depression and anxiety symptoms (Cook et al., 2019; Labelle et al., 2010). Mindfulness is an effective intervention shown to help people disengage from rumination (Falsafi, 2016; Perestelo-Perez et al., 2017). Practicing mindfulness encourages nonjudgmental focus on the perception and sensation of the present moment (Roca et al., 2021). Different modalities of a mindfulness intervention, such as a brief mobile app mindfulness intervention, are also effective in alleviating people's rumination and depressive symptoms (Hilt & Swords, 2021). Hence, I propose that a brief mindfulness mobile app intervention would lower rumination, subsequently causing people to have less pathogenic gut microbiota and more beneficial gut microbiota.

Although past studies have found the relationship between gut microbiota alteration and psychological disorders, no studies have investigated whether improvement in mental health can improve the gut microbiota composition. Thus, investigating the relationship

between improvement of rumination with a brief mindfulness mobile app and how it affects the gut microbiota composition is novel. Exploring the impact of a brief mindfulness mobile app on the gut microbiome is crucial because the mindfulness app intervention could be an accessible treatment for both gut and mental health in addition to conventional treatments.

There are no previous studies I could identify which investigated the influence of a brief mindfulness mobile app intervention alone on the gut microbiota. One past study has found that people who engaged in meditation and a vegan diet for more than three years had significantly different gut microbiome compositions than omnivorous people who had never engaged in any meditation training (Jia et al., 2020). They found that vegan people who consistently engaged in meditation had a lower abundance of Actinobacteria and Proteobacteria and an increased abundance of Firmicutes at the phylum level. They also found an increased abundance of *Roseburia* at the genus level. However, since Jia and colleagues (2020) investigated the influence of both meditation and vegan diets on the gut microbiota, it is unclear how meditation alone affects the gut microbiota. Moreover, their participants practiced meditation and a vegan diet for more than three years, so studying how a brief intervention influences the gut microbiome is warranted.

The current study investigated two hypotheses: 1. There is a positive correlation between the pre-intervention rumination score and the abundance of pathogenic/proinflammatory bacteria, whereas there is a negative correlation between the pre-intervention rumination score and the abundance of beneficial/anti-inflammatory bacteria and 2. engaging in four weeks of a brief mindfulness mobile app intervention increases the abundance of some beneficial gut microbiome and reduces the abundance of some pathogenic gut microbiome compared to before the intervention through the mediating effect of decreased rumination.

Methods

Participants

The participants were 16 first-year students at Lawrence University. First-year undergraduates were chosen as participants, because the transition to college is associated with heightened psychological stress due to the drastic change in their environments (Rayle & Chung, 2007). The inclusion criterion was being a first-year student at Lawrence University. The exclusion criterion was practicing meditation or mindfulness. I excluded 6 students from participating based on the exclusion criteria. Participants were 18.75% male, 50% female, 18.75% non-binary, and 12.5% did not categorize themselves. The self-reported race was 81.25% White, 12.5% Asian, and 6.25% multiracial. The self-reported ethnicity was 81.25% non-Hispanic, and 18.75% Hispanic (6.25% Mexican, 6.25% Dominican, 6.25% Central or South American).

Procedure

First-year undergraduates were recruited to participate in the study through posters, word-of-mouth, and announcements in introductory biology and psychology classes. Students who were interested in participating in the study scanned a QR code on the poster linked to the screening questionnaire to determine their eligibility to participate. Thirty-five students completed the screening questionnaire with Qualtrics survey software (Qualtrics, UT, USA). Sixteen first-year students were then chosen as participants based on the inclusion and exclusion criteria. They were invited to schedule a laboratory visit to proceed with the study. In the first lab visit, participants provided written consent and completed the baseline questionnaire using Qualtrics survey software, and installed the CARE app (Hilt & Swords, 2021) on their mobile device. They also received a stool sample collection kit, including a specimen collector pan and tube, to submit the sample. The participants submitted their pre-intervention stool sample within two days after they visited the lab. Upon submitting their pre-intervention stool sample, participants were notified to use the CARE app three times per

day for four weeks. At the end of four weeks, participants revisited the lab to complete the post-intervention questionnaire using Qualtrics survey software. They submitted their second stool sample within two days after the second lab visit. Participants received $20 for participation: $5 at the initial lab visit and $15 when they completed the study.

Of the 16 participants who were enrolled, 11 completed the post-intervention survey and 10 completed the entire study. There were no significant group differences in demographic variables or baseline variable between completers and those lost to follow-up, with the exception of the withdrawal of the two participants who did not fit into any of three gender categories I provided in the questionnaire. Although there might be a relationship between the participants who did not fit into any of the three gender categories and the reasons why they withdrew from the study, I could not detect a distinct trend given the small sample size.

Mobile App

Participants received reminder to use the app three times a day. Notifications were timed based on sleep and wake times that participants reported when they first downloaded the app during their lab visit (i.e., post-wake-up, afternoon and before bedtime) and were randomized. Each time participants opened the CARE app, they were asked nine questions regarding their current thoughts and rated their mood on sliding scales. If participants' sad or anxious mood rating was 90 or above (out of 100), they had an 85% chance of receiving a mindfulness exercise. If their rating was lower than 90, their chance of receiving an exercise was 67%. The app was made in this way so that participants would have a greater chance of engaging in a mindfulness exercise when they needed it to alleviate their sad or anxious moods while still preventing them from learning the pattern and providing their answer to get or avoid a mindfulness exercise. If a mindfulness exercise was given, participants were asked how much time they had from the range of 0-15 minutes and were randomly assigned an

exercise that fit within the time frame. The 1-minute exercises provided written instructions for focusing on physical sensations, sounds, or breath with a 60 second timer (Hilt & Swords, 2021). The 3- to 5- minute exercises provided guided audio for breathing, sounds, or body scans (i.e., tuning the awareness to the sensations in their body nonjudgmentally; Murphy et al., 2022). The 10- to 12- minutes exercises additionally provided other commons mindfulness exercises (Hilt & Swords, 2021). Participants engaged in this process for four consecutive weeks.

Outcome Measures

Trait Rumination. The Ruminative Response Subscale (RRS) was used to measure trait rumination (Nolen-Hoeksema & Morrow, 1991). The RRS is a 22-item self-report questionnaire which is summed to calculate the total score. The RRS total score measures an individual's ruminative tendency using a 4-point Likert scale (1 = *almost never*, 2 = *sometimes*, 3 = *often*, 4 = *almost always*). Sample items include, "Think about how alone you feel," "Think 'What am I doing to deserve this?'," and "Think about a recent situation, wishing it had gone better." This measure has shown good internal consistency and reliability (Lei et al., 2017). In this study, RRS showed good reliability of $\alpha = .802$ (pre-intervention) and $\alpha = .806$ (post-intervention).

Depressive Symptoms. The Beck Depression Inventory (BDI- II) was used to measure depressive symptoms (Beck et al., 1996). It is 21-item self-report items that use a 4-point scale ranging from 0 (*symptom absent*) to 3 (*severe symptom*). The BDI- II total score measures an individual's depressive symptoms over the past two weeks. Sample items include: "0 = *I do not feel sad*, 1 = *I feel sad much of the time*, 2 = *I am sad all the time*, 3 = *I am so sad or unhappy that I can't stand it*." High internal consistency for this measure has been reported (Beck et al., 1988). In this study, BDI- II showed adequate reliability of $\alpha = .766$ at pre-intervention and good reliability of $\alpha = .839$ at post-intervention.

Anxiety Symptoms. The Beck Anxiety Inventory (BAI) was used to measure anxiety symptoms (Beck et al., 1988). It is a 21-item self-report questionnaire that uses a 4-point scale (0 = *Not at all,* 1 = *Mildly, but it didn't bother me much*, 2= *Moderately, it wasn't pleasant at times,* 3 = *Severely, it bothered me a lot*). The BAI total score measures an individual's anxiety symptoms during the past month. Sample items include, "Unable to relax," "Fear of worst happening," and "Terrified or afraid." The BAI has been reported to have high internal consistency and test-retest reliability (Beck et al., 1988). In this study, BAI showed high reliability of α = .917 at pre-intervention and adequate reliability of α = .728 at post-intervention.

Worry. The Penn State Worry Questionnaire (PSWQ) was used to measure worry (Meyer et al., 1990). It includes 16-items that uses a 5-point Likert scale (1 = *not at all typical of me* to 5 = *very typical of me*). The total score of the PSWQ measures an individual's tendency to worry. Sample items include, "My worries overwhelm me," "I am always worrying about something," and "I worry about projects until they are all done." The PSWQ has demonstrated high internal consistency and good test-retest reliability (Meyer et al., 1990). In this study, PSWQ showed high reliability of α = .907 (pre-intervention) and α = .870 (post-intervention).

Trait Mindfulness. The Five Facet Mindfulness Questionnaire (FFMQ) was used to measure five dimensions of mindfulness (Baer et al., 2006). These five facets include observing, describing, acting with awareness, nonjudgment of inner experiences, and nonreactivity to inner experience. It contains 39 items and uses a 5-point scale (1 = *Never or very rarely true*, 2 = *Rarely true,* 3= *Sometimes true,* 4 = *Often true,* 5 = *Very often or always true*). The sample items are, "When I'm walking, I deliberately notice the sensations of my body moving," "I am easily distracted," and "I can usually describe how I feel at the moment in considerable detail." The FFMQ has shown adequate internal consistency (Shallcross et

al., 2020). In this study, the reliability for the subscales were as follows: Observing (pre-intervention α = .642, post-intervention α = .454), Describing (pre-intervention α = .902, post-intervention α = .879), Awareness (pre-intervention α = .791, post-intervention α = .805), Nonjudgment (pre-intervention α = .897, post-intervention α = .885), and Nonreactivity (pre-intervention α = .780, post-intervention α = .724). Since the reliability of Observing subscale was low, it was excluded from analyses.

Additional Information

Since research has shown that multiple factors influence the gut microbiome, I asked the participants to report some possible confounding variables that might also alter the gut microbiome and affect the results (Ianiro et al., 2016; Karl et al., 2018; Mach & Fuster-Botella, 2016; Mohajeri et al., 2018; Sánchez et al., 2017). In this study, I obtained information about participants' medication intake: whether they had taken any antibiotics within a year, any probiotics or probiotic supplements and its frequency, any form of anti-inflammatory medication (e.g., ibuprofen, acetaminophen, etc.) in the last three months, and any other medications such as dietary supplements. Regarding participants' diet, I also assessed their dietary restrictions (i.e., vegan, vegetarian, pescatarian, gluten-free, etc.), dietary habits (i.e., food groups they usually consume in each meal), and how often they consume alcohol. I also asked how often they exercise as well as whether they were receiving any treatments for a psychological disorder (e.g., anxiety, depression, ADHD, etc.) during the study. If they were doing so, they specified which type of treatment (i.e., medication, psychotherapy, or combination of both) and how often they partook in therapy.

Gut Microbial Community Collection

The stool sample was self-collected by the participants at a designated restroom in the same building as the laboratory. All the stool samples were collected within two days after the lab visit except for one baseline sample collected after 5 days. Participants collected their

stool sample using a specimen collector pan and submitted one scoop of stool sample using a DNA/RNA Shield Fecal Collection Tube (Zymo Research, CA, USA). Participants wore disposable gloves when they engaged in sample collection and were encouraged to wash their hands thoroughly after the sample collection. All the materials used during the sample collection were disposed of in a biohazardous waste container. I followed the Biosafety Level 2 protocol to avoid contamination by wearing lab coats and gloves when I retrieved the submitted samples. Additionally, I checked the designated bathroom that the participants underwent collection at least twice a day during the sample collection period and disinfected the surfaces. The submitted stool samples were stored in a freezer until all samples had been collected. Once all the samples were collected, I sent them to Zymo Research for microbial community analysis.

Ethical Consideration

The study was approved by the Lawrence University Institutional Review Board (IRB). The study protocol followed the IRB ethical guidelines and Biosafety Level 2 guidelines for safety. Participants who expressed interest in participation were introduced to the study details and signed the written informed consent forms.

Data Analytic Plan

Psychological Measures. To examine whether the brief mindfulness mobile app intervention improved participants' psychological health, I investigated how psychological variables (i.e., rumination, depression, anxiety, worry, and mindfulness) changed at post-intervention. I first examined skewness and kurtosis for all variables for psychological measures. Next, I investigated how much each variable value changed from pre-intervention to post-intervention with paired-samples *t*-tests to test the effect of the brief mobile app mindfulness intervention on psychological factors. For the variables that showed significant change, I ran multivariate tests with covariates to see whether the changes in such variables

were impacted by control variables (i.e., whether they take antibiotics, anti-inflammatory medication, or probiotic products, and whether they exercise). These analyses were conducted with SPSS (IBM, NY, USA). Because this was a preliminary study with small sample size, the confidence interval I used for data analysis was 90%, $p < 0.1$.

Gut Microbiome Analysis. The collected stool sample was analyzed by Zymo Research with 16S rRNA sequencing for microbial compositions, alpha diversity (i.e., Shannon index and Chao 1index), and beta diversity (i.e., unweighted and weighted UniFrac). To examine the relationship between rumination and the gut microbiome, I used the percentage abundance of the gut microbiota composition present in each sample at each taxonomy level from phylum to genus (i.e., phylum, class, order, family, genus).

Evaluating the Gut Microbiome and Relationship with Psychological Measures. To analyze the relationship between the rumination scores and the gut microbiome before the intervention, I ran Pearson bivariate correlation analyses between the pre-intervention rumination scores and the abundance of the gut microbiome detected in each sample from pre-intervention sample collection. To examine whether there was a significant change in the gut microbiome composition after the brief mindfulness intervention, I first tested the significant difference between each gut microbiota composition at each taxonomic level with paired-samples *t*-tests. For the genus found to significantly decrease or increase at post-intervention, and the direction of alteration was consistent with the prediction, I ran a mediation analysis using PROCESS macro (Model 4, Hayes, 2017) to see whether such changes were mediated through a decrease in the rumination score. The change in rumination score was attained by calculating residualized change scores. These analyses were conducted with SPSS.

Results

Psychological Outcomes

In examining skewness and kurtosis for each psychological variable, post-intervention depression showed high kurtosis (3.59). Skewness and kurtosis were in the normal range for other variables at both pre-intervention and post-intervention. I did not correct for the outliers because the present study is a preliminary study with a small sample size.

Paired-samples *t*-tests regarding participants' change in psychological factors with the brief mindfulness mobile app intervention are presented in Table 1. Paired-samples showed that participants' average rumination scores significantly decreased after the 4-week app intervention period with a large effect size. Average depression scores also significantly decreased from pre- to post-intervention with a large effect size. Average anxiety score decreased significantly after the intervention period with a moderate effect size. However, worry did not change. Three of the mindfulness facets—describing, nonjudgment of inner experiences, and nonreactivity to inner experience—increased at post-intervention. They all showed a large effect size. However, the mindfulness facet of acting with awareness did not increase at post-intervention.

Multivariate tests revealed no control variables (i.e., antibiotics, anti-inflammatory medication, probiotics intake, whether they exercise) impacted decrease in rumination. The decrease in depression and anxiety at post-intervention were also not impacted by the control variables. The increase in describing, nonjudgment of inner experiences, and nonreactivity to inner experience were not impacted by the control variables as well. Results of multivariate tests with control variables are presented in Table 2.

Gut Microbial Community

Alpha diversity, which is a metric that describes the amount of diversity present in any individual sample independently of all other samples, suggested each sample's diversity did not change significantly after engaging in the brief mindfulness mobile app intervention. These data were calculated using both Shannon index and Chao 1 index, which are metrics

that estimate the richness of taxa represented in a data set that are weighted/influenced based the respective abundance of taxa represented. The result of Shannon index is presented in Figure 1 and the result of Chao 1 index is presented in Figure 2. These results suggest that the amount of diversity present in any one individual's gut microbiota did not significantly alter during the 4-week intervention period.

Beta diversity shows the diversity of the gut microbiota composition in comparison to other individuals. The unweighted UniFrac that shows beta diversity irrespective of the abundance of each taxon relative to each sample, showed a couple of natural groupings that are represented as clusters. A visualization of this result is presented in Figure 3. However, the grouping was not based on pre-intervention rumination scores nor by pre-intervention and post-intervention samples. This suggests that there might be other factors created this grouping outside of my research interest for this study. Using a weighted UniFrac that incorporates the relative abundance of each taxon showed no distinct grouping of samples This result is visualized in Figure 4. This suggests that while certain taxa may be more/less represented in the fecal sample of certain individuals, this variability is less distinguishable if you take the abundance of sequence reads representing certain taxa into consideration.

Hypothesis 1

When analyzing the data with respect to broader taxonomic relationships to more specific ones, we only see one moderate but statistically significant correlation between pre-intervention rumination and the gut microbiome. At the broadest phylum level, there was no significant correlation observed between rumination score before the intervention and the gut microbiome composition. At an intermediate class level, there was also no significant correlation between pre-intervention rumination score and the gut microbiome. However, at a more defined family level, there was one moderate, but significant negative correlation

between pre-intervention rumination score and *Prevotellaceae* abundance (r = -.589, p = 0.027, df = 13).

A comparison of the data at the most targeted level of analysis was to the genus level and revealed three significant correlations when comparing the pre-intervention rumination scores and the gut microbiome. One of the taxa was in the family *Rikenellaceae*, but it has yet to be described at the more specific taxonomic level. Organisms within the genus *Adlercreutzia* abundance showed a moderate, but significant positive correlation with pre-intervention rumination score (r = 0.55, p = 0.042, df = 13). Finally, organisms within the genus *Prevotella* abundance had moderate, but significant negative correlation with pre-intervention rumination score (r = -0.57, p = 0.035, df = 13). Interestingly, only one of these three genera, *Prevotella*, belongs to the family *Prevotellaceae*, which also had significant negative correlation with pre-intervention rumination score whereas *Rikenellaceae* is a member of the Bacteroidales and *Aldlercreutzla* is a member of the Eggerthellales.

Hypothesis 2

Paired-samples *t*-tests regarding participants' change in abundance of gut microbiome after a brief mindfulness mobile app intervention are presented in Table 3. Paired-samples *t*-tests revealed significant change in abundance of two bacteria groups within the gut microbiome at the phylum level. First, the abundance of Actinobacteria increased significantly after the intervention. Additionally, the abundance of Firmicutes decreased significantly. Since both Actinobacteria and Firmicutes showed significant change after the intervention, all bacteria belong to these phyla were tested for their change at each taxonomic rank. There was a significant increase in class Actinobacteria and there was a significant decrease in class Clostridia. At the order level, I investigated the gut microbiome under class Actinobacteria and Clostridia. There was a significant increase in Bifidobacteriales and there was a significant decrease in Clostridiales. At the family level, *Bifidobacteriaceae* was

significantly increased and *Lachnospiraceae* was significantly decreased. At the genus level, *Bifidobacterium* was significantly increased and *Marvinbryantia* was slightly decreased. Furthermore, genus *Alistipes* increased significantly.

To investigate whether the significant change in *Bifidobacterium* and *Marvinbryantia* occurred indirectly through the decrease in rumination score, I ran the mediation analysis. A visualization of the mediation analysis for *Bifidobacterium* is presented in Figure 5, and a visualization of the mediation analysis for *Marvinbryantia* is presented in Figure 6. I did not run a mediation analysis for increased *Alistipes* abundance because it contradicts to the prediction. The mediation analysis revealed that the abundance of pre-intervention *Bifidobacterium* predicted the rumination change, but the rumination change did not predict the abundance of *Bifidobacterium* after the intervention. The abundance of pre-intervention *Marvinbryantia* did not predict the rumination change, and the rumination change also did not predict the post-intervention *Marvinbryantia* abundance. Thus, there was no support for an indirect effect through rumination in increasing *Bifidobacterium* abundance and decreasing *Marvinbryantia* abundance.

Discussion

The goal of this study was to investigate the relationship between rumination and the gut microbiome as well as the impact of a brief mindfulness mobile app intervention on the gut microbiome. There were moderate but significant correlations between pre-intervention rumination and three gut microbiome groups. However, those results were inconclusive because there were not enough past studies to know whether those bacteria groups are pathogenic or beneficial. Future studies need to investigate the relationship between rumination and the gut microbiome with a bigger sample to detect trends more accurately. Additionally, this study observed a potential effect of the brief mindfulness mobile app to positively impact the gut microbiome, but there was no evidence that it occurred indirectly,

through an improvement in rumination. Future studies with randomized controlled trials are needed to draw a conclusion for the efficacy of the intervention on the gut microbiome as well as the mechanisms of how the gut microbiome alteration may occur with the intervention.

Manipulation Check

I first checked the brief mindfulness mobile app worked as expected by examining its effect on psychological health. The results showed that rumination, depression, and anxiety decreased, and describing, nonjudgment, and nonreactivity of mindfulness subscale increased after the 4-week intervention. These results suggest that the brief mindfulness mobile app intervention appeared to have the intended effect of improving individuals' psychological health.

Gut Microbial Diversity

Alpha diversity analyzed with Shannon and Chao 1 index revealed that there was no significant change in diversity in samples during the 4-week intervention period. Unweighted UniFrac analysis showed natural grouping of samples, but the grouping was based on neither rumination score nor the sample collection time points. The grouping may have occurred based on the presence and absence of specific gut microbiota taxa, but further investigation is needed to evaluate this possibility. Weighted UniFrac analysis showed even less groupings of samples, suggesting that the abundance of gut microbiota taxa varies between samples regardless of the sample collection time points. Thus, measures of alpha and beta diversity did not resolve any specific changes in microbial diversity that were correlated with rumination score or during the intervention period.

Hypothesis 1: Rumination and the Gut Microbiome

I tested the hypothesis that there is a positive correlation between the rumination score and the abundance of proinflammatory/pathogenic bacteria, whereas there is a negative

correlation between the rumination score and the abundance of anti-inflammatory/beneficial bacteria. This study observed a correlation between pre-intervention rumination and a bacteria group at the family level and two correlations between rumination and bacteria groups at the genus level. The results were inconclusive because whether the gut microbiome groups are pathogenic or beneficial is not clear from the past studies. Additionally, this study is limited in its ability to find trends between rumination and the gut microbiome groups due to its small sample size.

At the family level, there was a moderate but significant correlation between *Prevotellaceae* and pre-intervention rumination score. At the genus level, *Prevotella*—a genus under the family *Prevotellaceae*—and pre-intervention rumination score had a moderate but significant negative correlation. These correlations were moderately strong, which is impressive given that they are between psychological and biological variables (Martínez et al., 2012). *Prevotella* is a common gut microbiota genus seen in individuals who consume a plant-rich diet high in fiber and carbohydrates. Therefore, it may be associated with beneficial effects for the host (Ley, 2016; Precup & Vodnar, 2019). If this was the case, the hypothesis is supported because this study observed a negative correlation between pre-intervention rumination and *Prevotella,* a possible beneficial bacteria group.
However, *Prevotella* is also related to increased inflammation, and its increased abundance is also reported in HIV patients (Ley, 2016). Moreover, a past study focused on comorbid IBS with depression and anxiety reported that individuals with comorbid IBS had increased *Prevotella* abundance (Simpson et al., 2020). These results suggest that *Prevotella* is pathogenic bacteria. If this was the case, the hypothesis is not supported. Due to the inconsistency in the findings on *Prevotella* in past research, I cannot conclude whether the genus *Prevotella* is a beneficial or pathogenic bacteria and whether the negative correlation

observed between pre-intervention rumination and *Prevotella* abundance supports the hypothesis.

Furthermore, in this study, the genus *Adlercreutzia* was positively correlated with the pre-intervention rumination score. *Adlercreutzia* belongs to the family *Eggerthellales*, which did not correlate with the pre-intervention rumination score. Xu and colleagues' study (2018) suggested a possible beneficial feature of *Adlercreutzia*, finding that *Adlercreutzia* was negatively correlated with anxiety-like behavior in mice. However, their study focused on alcohol addiction, not depressive-like behavior, and used mice as subjects as opposed to humans. Thus, their findings are not generalizable to support the possible beneficial feature of *Adlercreutzia* and whether the result contradicts to the hypothesis.

Another study indicated the genus *Adlercreutzi* may have beneficial properties. It suggested that the genus *Adlercreutzia* participated in brain inflammatory signaling in a multiple sclerosis study with human subjects (Chen et al., 2016). In particular, *Adlercreutzia* was less abundant in relapsing remitting multiple sclerosis patients. This result suggests *Adlercreutzia*'s potential beneficial role in neuroimmune regulation (Chen et al., 2016). If this was the case, the result found in this study contradicts the hypothesis. However, while the above study indicates that the genus *Adlercreutzia* may be a beneficial bacteria genus in the gut, not enough research has been conducted on humans to make a definitive conclusion. Therefore, I cannot conclude whether this result was contradictory to our hypothesis.

Moreover, aforementioned correlations between pre-intervention rumination and the gut microbiota groups were run with 14 subjects. Result of studies with a small sample size are more likely to be affected by individual differences. Because I ran multiple correlations, the correlations in this study may have been found by chance (i.e., a Type 1 error). Also, this study may have missed some existing correlations due to the lack of power to detect the small

effects because of the small sample size (i.e., a Type 2 error). Future studies should investigate the relationship between rumination and the gut microbiome with a bigger sample size to determine whether rumination is reliably correlated with certain gut microbiota composition.

Hypothesis 2: The Mindfulness Intervention and the Gut Microbiome

I also tested the hypothesis that engaging in 4 weeks of a brief mindfulness mobile app intervention increases the abundance of beneficial gut microbiota taxa and reduces some pathogenic gut microbiota taxa abundance compared to before the intervention through the mediating effect of rumination change. This hypothesis was partially supported by the results. The paired-samples *t*-test revealed that the abundance of genus *Bifidobacterium* significantly increased after the brief mindfulness mobile app intervention. *Bifidobacterium* is a well-known beneficial genus of bacteria that is often contained in probiotics products such as fermented milk products, kimchi, and kombucha. Past studies have found that *Bifidobacterium* is associated with a reduction in depressive symptoms (Okubo et al., 2018; Pinto-Sanchez et al., 2017). This suggests that the genus *Bifidobacterium* is a beneficial bacteria genus and that its abundance might have increased due to engaging in a brief mobile app mindfulness intervention.

The paired samples *t*-test also revealed that the abundance of genus *Marvinbryantia* was decreased after the brief mindfulness intervention. Not many studies have been done to investigate the characteristics of the genus *Marvinbryantia* with human subjects, but some studies with rodents have suggested that *Marvinbryantia* is a pro-inflammatory gut microbiota (Wang et al., 2020). For example, a study with mice found that the abundance of *Marvinbryantia* increased after traumatic brain injury, which induces neuroinflammatory responses (Treangen et al., 2018). Additionally, another study found that chronic mild stress significantly increases the abundance of *Marvinbryantia* (Xu et al., 2022). The genus

Marvinbryantia is also associated with obesity (Clarke et al., 2012). The aforementioned studies thus suggest that *Marvinbryantia* is a pathogenic bacteria genus. Therefore, the decrease in *Marvinbryantia* in the present study with the brief mindfulness mobile app intervention would be considered beneficial.

While the abundance of one pathogenic taxon, *Marvinbryantia*, decreased after the intervention, the abundance of another pathogenic genus, *Alistipes*, increased after the brief mindfulness mobile app intervention. In past studies, abundance of *Alistipes* was increased in people with MDD (Jiang et al., 2015; Naseribafrouei et al., 2014). Therefore, I expected that this genus would decrease after the mindfulness intervention, but the result was contradictory. A randomized controlled study with a bigger sample size is needed to test whether the increase in *Alistipes* following a brief mindfulness intervention is accurate to ensure that the brief mindfulness mobile app intervention does not negatively impact the gut microbiome.

Although one pathogenic gut microbiota genus increased after the intervention, a brief mobile app mindfulness intervention may still be effective in contributing to increasing certain beneficial bacteria (i.e., *Bifidobacterium)* and decreasing pathogenic bacteria (i.e., *Marvinbryantia*). However, the current study was not a controlled study, so I cannot conclude that the brief mindfulness mobile app intervention alone, and no other confounding factors (i.e., diet, probiotic intake, the passage of time, etc.) caused such changes in the gut microbiota composition. Therefore, future studies should conduct a randomized controlled trial with a larger sample size to examine whether the abundance of *Bifidobacterium* increases and the abundance of *Marvinbryantia* decreases with the brief mindfulness mobile app intervention alone.

The current study did not conclude that the change in those gut microbiota compositions was mediated by rumination. The abundance of genus *Bifidobacterium* before

the intervention predicted the change in rumination, but the change in rumination did not predict the abundance of post-intervention *Bifidobacterium.* This result suggests that there might be other factors that influenced the change in *Bifidobacterium* abundance. One of the possible ways *Bifidobacterium* abundance alterations may have occurred is through multiple pathways of GBA and with other mechanisms. One of the possible routes of GBA influencing *Bifidobacterium* abundance is through the mindfulness mobile app working on the endocrine system. Studies have found that engaging in mindfulness meditation can reduce cortisol levels in the host's system (Turakitwanakan et al., 2013). This decrease in cortisol level may be related to the increase in *Bifidobacterium* abundance. In past research, a significant negative correlation was found between the abundance of *Bifidobacterium* and cortisol levels, suggesting the abundance of *Bifidobacterium* may increase with a decrease in cortisol level by the mindfulness intervention (Aizawa et al., 2018). The effects of cortisol level change on the gut microbiome abundance should be investigated in future studies. Future studies should also explore other GBA pathways which may involve *Bifidobacterium* abundance change to examine how the brief mindfulness mobile app intervention may affect the gut microbiota.

Furthermore, the pre-intervention abundance of genus *Marvinbryantia* did not predict rumination change, and neither did the rumination change predict the post-intervention *Marvinbryantia* abundance. This result suggests that there might be also other factors that influenced the change in *Marvinbryantia* abundance. There is no past research to indicate the possible mechanism of how *Marvinbryantia* abundance may change with a mindfulness intervention. Therefore, future studies should also explore how *Marvinbryantia* abundance alteration may occur.

Limitations

One major limitation of the current study was the small sample size. This small sample size made it harder to accurately detect the relationship between rumination and the gut microbiota compositions as well as the effects of gut microbiota change over time. Attrition also occurred, further limiting the sample size for my second hypothesis. I started the study with 16 participants, and I lost many participants to follow-up. Six out of 16 participants did not complete the post-intervention sample collection. This significant loss of participants may be due to two reasons. Firstly, participants might have felt uncomfortable returning for the second sample collection. Although I informed the participants that they will have two stool sample collections and they have signed the informed consent to agree with their participation, the stool sample collection might be something more uncomfortable than they expected. Secondly, the majority of the participants engaged in the second sample collection during the final exam period, and some of them might not have had time to collect and return their sample. Additionally, the majority of participants I lost started the study later than others, which might add extra time constraints on them as they needed to prepare to leave the campus for break.

In addition to the small sample size, it is also important to mention that the gut microbiota composition is unique to an individual. Such significant individual differences in the gut microbiota composition might have also made it difficult to detect trends. The gut microbiota composition can be influenced by a variety of factors including diet, exercise, and medications, and I could not control for all the variables. Therefore, differences between individuals might have impacted the results, making the overall trend difficult to interpret, especially in this small study. It warrants a bigger sample size in future studies to detect the accurate correlations between rumination and the gut microbiome, and a randomized controlled trial to maximize internal validity regarding the effect of the intervention on microbiota composition alterations.

Strengths

Despite the limitations, there are some strengths of this study worth being replicated in future studies. First, this study is the first to investigate the relationship between rumination and gut microbiome. Past studies have only focused on examining the relationship between psychological disorders and the gut microbiota in a clinical sample. Thus, the present study is the first step towards exploring how gut microbiota alterations may occur with the deterioration and improvement of an individual's mental health outside of a clinical context. Furthermore, the present study is the first to explore how gut microbiota composition improves with engagement in a brief mindfulness mobile app intervention. Despite the small sample size and lack of a control group limiting the conclusions that can be drawn, I found some possible trends of positive changes in the gut microbiota that should be investigated further.

Future Directions

To aid in prevention efforts for both mental and gut health, future studies should first further explore the relationship between rumination and the gut microbiota composition to check whether the gut microbiota change is occurring before psychopathology symptoms emerge. They should utilize a larger sample size to be able to detect the correlations between the gut microbiota compositions and the rumination scores more accurately. Furthermore, although I believe the present study helped some first-year students to mitigate their rumination, depression, and anxiety, future studies do not need to focus on first-year students in order to have more participants in the study to increase the generalizability of the outcomes.

Additionally, future studies should take the academic calendar into account to avoid confounding variables. For example, the students could be busier and more stressed than usual during the final exam period. Such extra stress among students during the specific

period may conceal the effect that occurred with the intervention if the data collection was conducted during such times. Therefore, future studies should avoid the exam period to avoid the impact on the data collection.

To examine how the brief mindfulness mobile app intervention alone influences the gut microbiota composition, future studies should conduct randomized controlled trials. The control group could use an app (e.g., to record their feelings, to note what they ate today, etc.), but not engage in the mindfulness intervention. This would control for various confounding variables including placebo effect and passage of time. Moreover, I recommend additional time points for data collection of psychological factors between pre-intervention and post-intervention time points to see whether the decrease in rumination score mediated the change in gut microbiota compositions. By having another time point to measure psychological factors, future studies can conduct a more accurate mediation analysis to see whether the decrease in rumination with the mindfulness intervention improves the gut microbiota composition (Maxwell & Cole, 2007).

Conclusion

The present study was a preliminary study that explored the relationship between rumination and the gut microbiome as well as the impact of a brief mindfulness mobile app intervention on the gut microbiome through change in rumination. Despite the small sample size, the brief mindfulness mobile app intervention showed its intended effect to improve mental health in this study. However, whether higher rumination is related to an individual's gut microbiome was inconclusive from this study due to the small sample size and inconsistency in past findings to support observed correlations. Additionally, the present study found that the abundance of a beneficial bacteria group *Bifidobacterium* increased and a pathogenic bacteria group *Marvinbryantia* decreased after the 4-week brief mindfulness mobile app intervention. These alterations were consistent with my prediction. However, the

alterations in these two bacteria groups were not mediated by change in rumination, suggesting the bidirectional relationship between the gut and psychological health may be intricately mediated by multiple factors. Moreover, the abundance of pathogenic bacteria group *Alistipes* increased after the intervention, which was opposite of what I predicted. Future studies should have a bigger sample size to investigate whether rumination is a factor that impacts an individual's gut microbiome. Future studies should also explore whether the brief mindfulness mobile app intervention alone affects the bacteria groups that observed the alteration in this study with a randomized controlled study.

The present study is crucial because it was the first study that explored the relationship between rumination and the gut microbiome. The current study is also vital because it was the first study that examined the efficacy of a brief mindfulness mobile app intervention in improving the gut microbiome composition with a decrease in rumination. This preliminary study would be the first step in exploring the pieces of the complex relationship between the gut microbiome and mental health. Additionally, the current study also offered valuable information on how a brief mindfulness mobile app not only reduces rumination but also could positively impact gut health which is worth further investigating in future studies.

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Table 1

Paired-Sample t-*test Results*

Outcome	Pre-intervention (N = 16)		Post-intervention (N = 11)		t	*p*	Cohen's *d*
df = 10	M	SD	M	SD			
Rumination	53.69	9.18	49.09	8.8	3.08	0.012**	0.93
Depression	41.38	8.29	32.91	7.64	4.62	<0.001***	1.39
Anxiety	41.31	13.37	33.64	6.35	2.1	0.062*	0.63
Worry	58.13	12.53	53.82	10.21	1.16	0.274	0.35
Describing (FFMQ)	22.69	7.08	25.55	6.98	-1.99	0.075*	-0.60
Awareness (FFMQ)	21.13	5.15	23.74	4.67	-1.5	0.164	-0.45
Nonjudgment (FFMQ)	22.13	7.75	27.27	7.3	-3.29	0.008***	-0.99
Nonreactivity (FFMQ)	18.94	5.6	20.91	4.78	-2.23	0.05*	-0.65

Note. * p <.1, ** p <.05, *** p <.01

Table 2

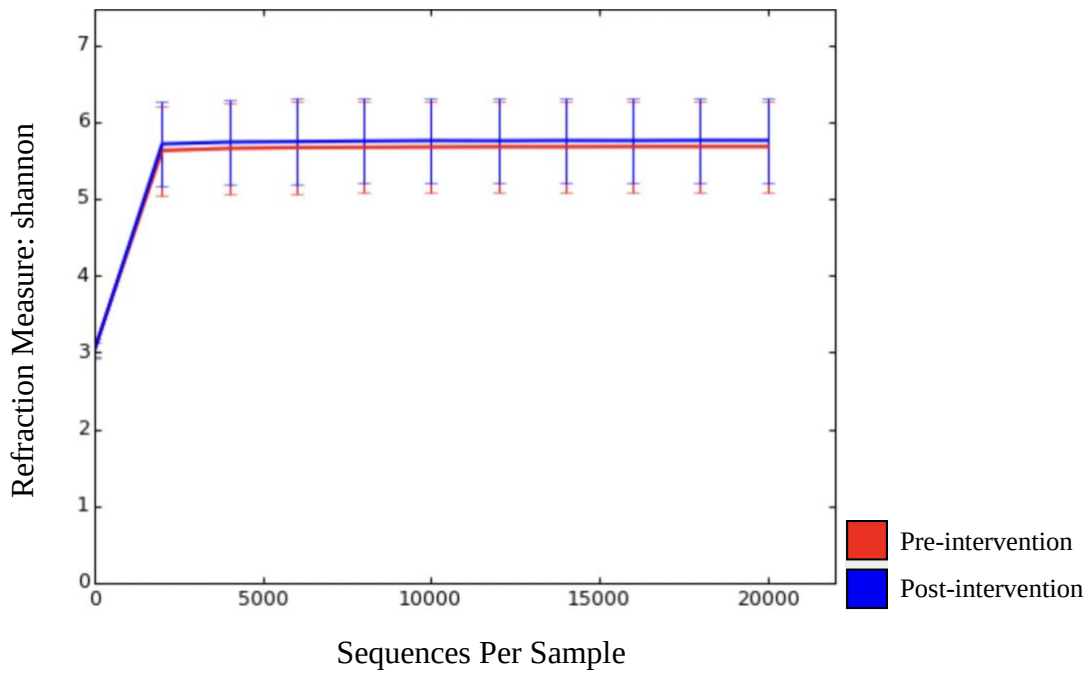
Multivariate Tests with Control Variables

Outcome *df* = 6	Rumination		Depression		Anxiety		Describing (FFMQ)		Nonjudgment (FFMQ)		Nonreactivity (FFMQ)	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Antibiotics	.230	.648	.173	.692	.252	.633	.049	.832	.001	.983	1.083	.338
Anti-infl. Med.	1.15	.324	.794	.407	1.850	.223	.016	.904	.510	.502	.060	.814
Probiotics	.491	.510	.040	.849	.009	.928	.002	.963	.150	.712	.003	.960
Exercise	3.273	.120	.012	.916	.003	.958	6.37	.045**	.347	.577	.493	.509

Note. * p <.1, ** p <.05, *** p <.01

Figure 1

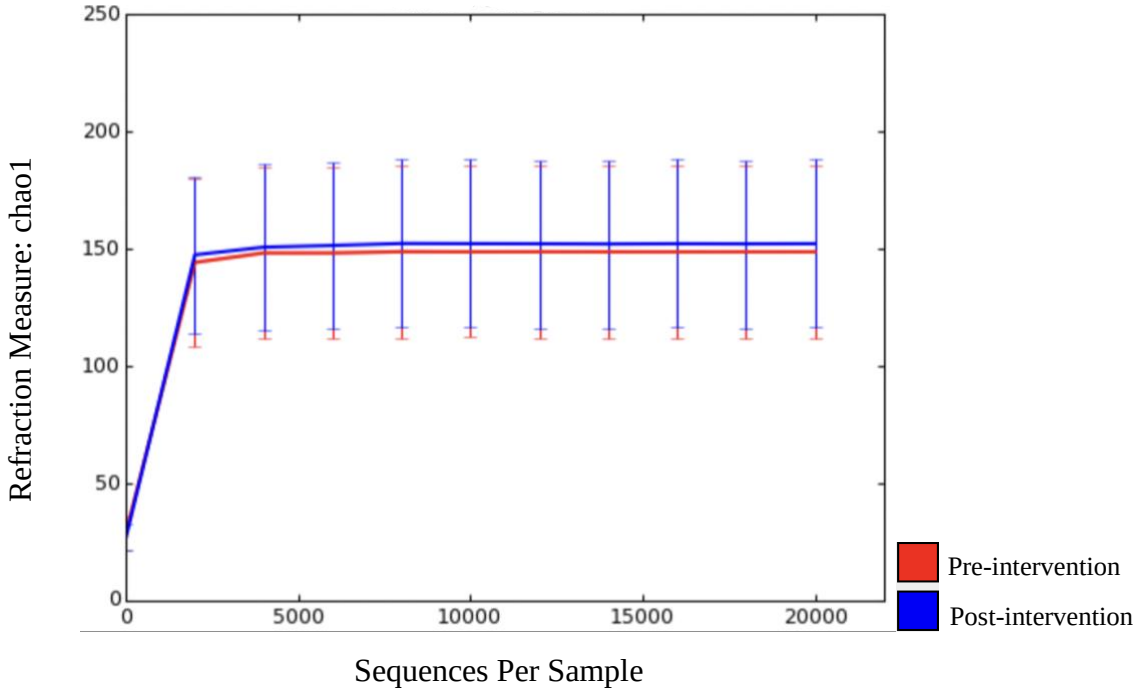
Alpha Diversity Analysis with Shannon Index Value



Note. The x axis shows that diversity is not correlated with the number of sequence reads after a threshold of approximately 3,000 sequence reads have been analyzed. The y axis shows the diversity of species exist in samples. The higher number indicates the greater diversity. The vertical lines show error bars.

Figure 2

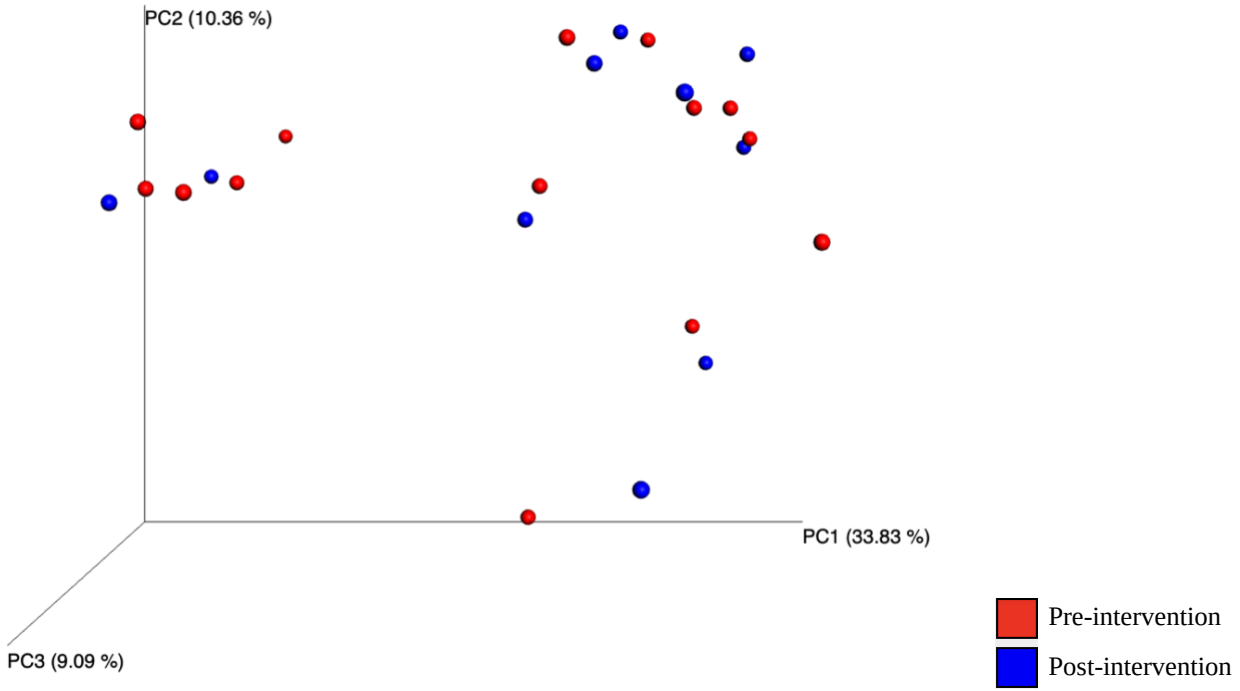
Alpha Diversity Analysis with Chao1 Index



Note. The x axis shows that diversity is not correlated with the number of sequence reads after a threshold of approximately 3,000 sequence reads have been analyzed. The y axis shows number of species exist in samples. The vertical lines show error bars.

Figure 3

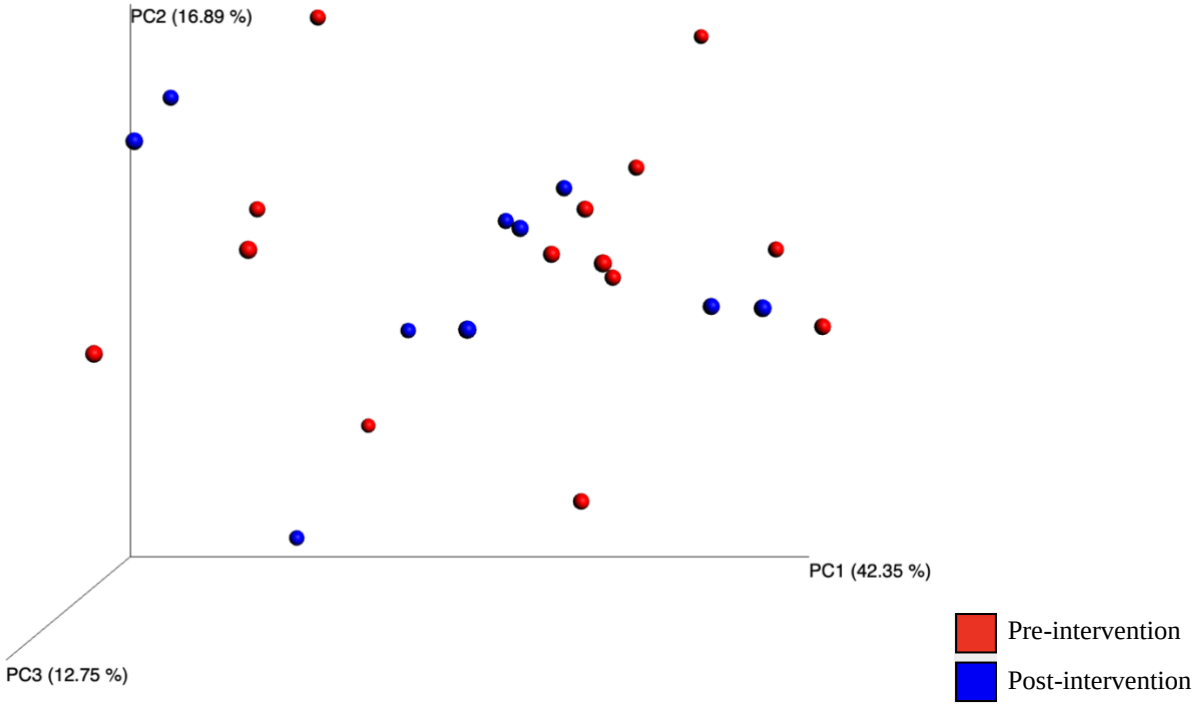
Beta Diversity Analysis with Unweighted UniFrac.



Note. Unweighted UniFrac shows the variability in types of species exist between the samples. Each red dot represents each participant at pre-intervention, and each blue dot represents each participant at post-intervention. Samples have similar species composition plotted close to each other.

Figure 4

Beta Diversity Analysis with Weighted UniFrac



Note. Weighted UniFrac shows the variability in the abundance of species that exist between samples. Each red dot represents each participant in pre-intervention, and each blue dot represents each participant in post-intervention. The samples with similar abundances of species showing the most variability is plotted close together.

Table 3

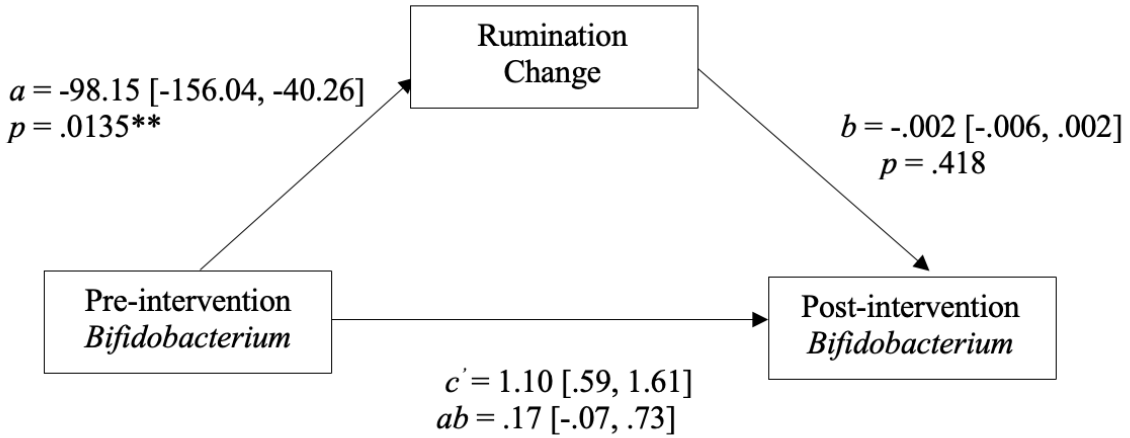
The Gut Microbiome Alteration after the Brief Mindfulness Mobile App Intervention at Each Taxon

Gut microbiome *df = 9*	t	*p*
	Phylum Level	
Actinobacteria (Increased)	.346	.062*
Firmicutes (Decreased)	5.569	< .001***
	Class Level	
Actinobacteria (Increased)	-2.413	.039**
Clostridia (Decreased)	4.479	.002***
	Order Level	
Bifidobacteriales (Increased)	-2.420	.039**
Clostridiales (Decreased)	4.479	.002***
	Family Level	
Bifidobacteriaceae (Increased)	-2.420	.039**
Lachnospiraceae (Decreased)	3.268	0.01**
	Genus Level	
Bifidobacterium (Increased)	-2.420	.039**
Marvinbryantia (Decreased)	1.849	.098*
Alistipes (Increased)	-2.309	.046**

Note. * p <.1, ** p <.05, *** p <.01

Figure 5

Bifidobacterium Mediation Analysis

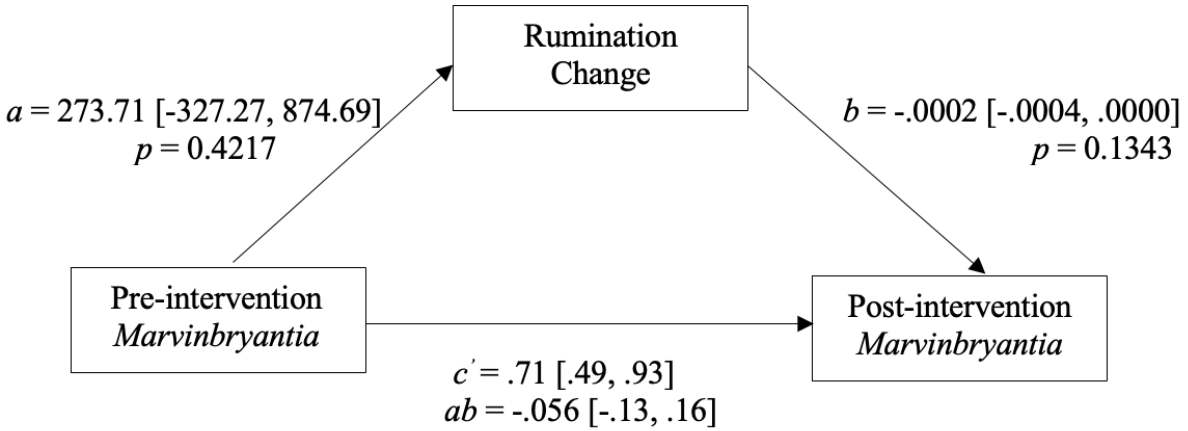


Note. a indicates the effect of pre-intervention *Bifidobacterium* abundance on rumination change. *b* indicated the effect of rumination change on post-intervention *Bifidobacterium* abundance. *c'* indicates the direct effect of pre-intervention *Bifidobacterium* on post-intervention *Bifidobacterium*. *ab* indicates the indirect effect of pre-intervention *Bifidobacterium* abundance on post-intervention *Bifidobacterium* abundance through rumination.

* $p < .1$, ** $p < .05$, *** $p < .01$

Figure 6

Marvinbryantia Mediation Analysis



Note. a indicates the effect of pre-intervention *Marvinbryantia* abundance on rumination change. *b* indicated the effect of rumination change on post-intervention *Marvinbryantia* abundance. *c'* indicates the direct effect of pre-intervention *Marvinbryantia* on post-intervention *Marvinbryantia*. *ab* indicates the indirect effect of pre-intervention *Marvinbryantia* abundance on post-intervention *Marvinbryantia* abundance through rumination.