Acute Supplementation with a Multistrain and Multispecies Probiotic Does Not Improve Gastrointestinal Symptoms, Depression and Anxiety in Young Adults: A Randomized, Double-Blind, Placebo-Controlled Trial



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Abstract

Depression, anxiety, and gastrointestinal discomfort are comorbid conditions with high prevalence and, surprisingly, have proven difficult to effectively treat. The gut-brain axis is the term used to explain the connection between the gut microbiome and the nervous system and provides a possible explanation for these comorbidities. Studies using animal models have revealed a link between gut microbiome alterations and anxiety, suggesting that gut microbes interact bidirectionally with other body systems, especially the nervous system. There is few, rigorous, prospective research examining these effects in humans. In this double-blind, placebo-controlled trial, one-hundred participants were randomly assigned to ingest two capsules, either VSL#3, a multistrain and multispecies probiotic, or a matched placebo, every day for 2 weeks. Participants completed the Center for Epidemiological Studies Scales of Depression, the State-Trait Anxiety Inventory, and the Gastrointestinal Symptom Rating Questionnaire before and after treatment. It was hypothesized that participants in the probiotic group would have significantly decreased depression, anxiety, and GI symptoms when compared to the placebo at Time 2. However, results indicated no significant interaction between time and treatment groups. These findings suggest that a 2-week supplementation with probiotics does not decrease depression, anxiety, or GI symptoms in young adults. Future research should further examine strain specific probiotics, and synergistic effects for increasingly longer durations to better understand how supplemental bacteria impact the immune, nervous, and GI systems.

Acute Supplementation with a Multistrain and Multispecies Probiotic Does Not Improve Gastrointestinal Symptoms, Depression and Anxiety in Young Adults: A Randomized, Double-Blind, Placebo-Controlled Trial

Major depression has a lifetime prevalence of 29.9% in the United States (Kessler, Petukhova, Sampson, Zaslavsky, & Wittchen, 2012); however, psychologists, psychiatrists, and neurologists have struggled for decades to identify the most effective way to treat depression and other mood and anxiety disorders. Mood and anxiety disorders are often comorbid with other symptoms that affect quality of life such as irritable bowel syndrome (IBS) and other Gastrointestinal (GI) symptoms (Lee et al., 2015; Whitehead, Palsson, & Jones, 2002). Researchers have analyzed numerous clinical trials submitted to the FDA for antidepressant pharmaceuticals and found that effectiveness, when compared to a placebo, was positively correlated with initial severity of depression. Participants with mild to moderate depression showed similar change in depressive symptoms in both the treatment and placebo groups. Common pharmacological treatments, including selective serotonin reuptake inhibitors (SSRIs) are no more effective in treating mild to moderate depression than placebos (Kirsch et al., 2008; Khan, Leventhal, Khan, & Brown, 2002), leaving many without long-term relief.

SSRIs may be targeting incorrect causes of mood and anxiety disorders, which could explain their inefficiency. A recent shift to viewing depression as an inflammatory and possibly an infectious disease (Canli, 2014; Devorak et al., 2015; Dowlati et al., 2010; Messay, Lim, & Marsland, 2012; Raison, Capuron, & Miller, 2006) suggests a shift in the field away from embracing the monoamine model of

depression. This could lead to more effective therapeutic treatments for depression and its comorbidities. Initial research on the gut microbiome theory and the hygiene hypothesis has shown that systemic inflammation is influenced by the composition of gut microbiota (Bercik et al., 2011; Dinan & Cryan, 2012; Olszak et al., 2012; Rook, Lowry, & Raison, 2013). It is possible that there is a connection between mood and anxiety disorders and the gut microbiota via inflammatory processes.

The Gut-Brain Axis

The gut-brain axis (GBA) is a term used to describe the bidirectional communication pathway that exists between microbes and their host's bodily systems. This axis is comprised of commensal microbes, the nervous system, specifically the central nervous system (CNS), the autonomic nervous system (ANS), and the enteric nervous system (ENS), as well as the endocrine and immune systems (Cryan and Dinan, 2012). The effects of the CNS on the ENS have been known for a long period of time and explains one of the many pathways involved in this system. The CNS signals to the ENS via the autonomic nervous system as well as serotonergic, noradrenergic and opioidergic descending spinal pathways and affects the motor, sensory, and immune functions of the GI system (Mayer, 2011). The gut then sends signals to various areas of the CNS via enteric afferent nerves that are stimulated by luminal conditions, mechanical stimuli, or signaling molecules such as cytokines or neurotransmitters. Neuropeptides and hormones can also signal to the CNS via an endocrine pathway (Mayer, 2011).

In addition to the ENS, the gastrointestinal tract contains 40,000 different species totaling over 10^{14} microorganisms (Frank & Pace, 2008) referred to as gut

microbiota. These organisms as well as the microbiota's genes, metabolites and their environment are collectively known as the microbiome. Many of the organisms have mutualistic relationships with their hosts, and influence not only the gastrointestinal system and the arrangement and function of the ENS (Collins, Borojevice, Verdu, Huizinga & Ratcliffe, 2014; Hoveyda et al., 2009; Kunze et al., 2009), but also the nervous (Bercik et al., 2011; Bravo et al., 2011) and immune systems (Leyer, Mubasher, Reifer & Ouwehand, 2009). Although adequate knowledge of the mechanisms that mediate the complex effects of the microbiome on its hosts and their organ systems is lacking, researchers have proposed a few that involve neurotransmitters and their receptors, metabolites produced by the bacterium interacting with the nervous system, and altered levels of pro-inflammatory cytokines in the immune system (Ivanov and Littman 2011; Kunze et al., 2009; Messaoudi et al., 2010; Tillisch et al., 2013).

The emerging theory surrounding the GBA states that the absence, presence, or alteration of the gut microbiome can cause changes in the brain, and has been supported by numerous animal studies (Bercik et al., 2011; Collins, Kassam & Bercik, 2013; Heijtz et al., 2011; Messaoudi et al., 2010; Schmidt et al., 2014). Fecal transplantation is a specific experimental manipulation that has contributed to this body of knowledge. Collins et al. used two strains of laboratory mice with different anxious behaviors and compared groups that were either gavaged their own feces or the feces of the opposite strain of mice (2013). When the NIH Swiss mice, which are naturally gregarious and less fearful, were fed the feces of BALB/c mice, a more anxious strain, they became anxious, evident by an increase in latency to step down

when compared to their controls. Inversely, when the BALB/c mice were gavaged with the feces from the NIH Swiss mice, their latency to step down decreased in comparison to the BALB/c mice that were gavaged with their own feces or no feces (Collins et al., 2013). This study has yielded results that suggest causality between the gut microbiome and animal behavior.

The Microbiota and the Immune System

The increase in research on the gut-brain axis has provided further insight into an older theory about microorganisms in the body, known as the hygiene hypothesis (Penders et al., 2014; Rook et al., 2013). This hypothesis is centered on the idea that exposure to microorganisms, especially early in life, plays a crucial role in the development and functioning of the healthy immune system. Exposure to pathogens early in development, which is often a factor of mode of birth and childhood environment, affects the immune system and its inflammatory responses (Rook et al., 2013). Natural early exposure to microorganisms, such as being raised in a rural versus urban setting, is associated with decreased likelihood of developing allergies (Björkstén, 2004; Leynaert et al., 2001; Penders et al., 2014), and other inflammatory disorders involving the immune system (Riedler et al., 2001; Strachan, 1989).

Disorders involving the immune system are often associated with a disruption of its regulatory mechanisms. The microbiome has been shown to not only influence crucial programming of a healthy immune system at birth, but also affect the regulatory mechanisms used by the immune system (Rook et al., 2013). This has been observed in the intestines as various microbes signal to specific

immune cells and to tight junctions of epithelial cells therefore affecting their cellular integrity and the immune response in the intestines (Ivanoc and Littman 2011). It is has been found that specific commensal bacteria also play a crucial role in the suppression of the immune system and regulation of Th2 cells in response to certain antigens so that the body can tolerate foreign substances such as food (Sudo et al., 1997). Therefore the composition of the microbiota may lead to an increase in the intestinal immune response and therefore intestinal inflammation leading to various GI symptoms.

Further evidence also suggests that the gut microbiome affects the immune response, specifically to illness and vaccines later on in life (Leyer et al., 2009; Oh et al., 2014; Olszak; 2012). These findings indicate the existence of a continuous and reciprocal interaction between the intestinal microbiome and the responsiveness of the immune system. Oh et al. found that plasma cell differentiation, a key factor in antibody production, was promoted by sensing the flagellin of bacteria by toll-like 5 receptors (TLR) found on cell surfaces that respond to bacterium (2014). Therefore, relative bacterial composition within the body and the organism's response to it plays a role in the immune system's response to outside pathogens. Olszak et al. further supported this idea by controlling the birthing environment of mice to either be "germ-free" (GF) or "specific pathogen free" (SPF) in order to induce differing microbiota between the mice (2012). Researchers found higher levels of invariant natural killer T (iNKT) cells, which are proinflammatory, in the colon and lungs of GF mice when compared to SPF mice (Olszak et al., 2012). Desbonnet, Garrett, Clarke, Bienenstock & Dinan, also found that mice treated with *Bifidobacteria*

infantis for 14 days had attenuating levels of three various proinflammatory cytokines when compared to their controls (2009). This could indicate that microbes placed in the SPF mice or in the probiotic treatment decreased pro-inflammatory cytokine levels which could be beneficial by leading to an overall decrease of inflammation in the host.

Although there are fewer human studies on the microbiome and the efficacy and responsiveness of the immune system, Leyer et al. studied a cohort of Canadian children 3-5 years of age (2009) and found that altering the microbiome using three different probiotics of the *Bifidobacterium* and *Lactobacillus* genera given twice daily for 6 months led to a reduced duration and incidence of fever, rhinorrhea, cough, and the use of antibiotics when compared to the placebo group. Children in the treatment groups, given two different cocktails of probiotics, also exhibited a significantly lower number of absences from child care (Leyer et al., 2009). Accumulation of more clinical evidence in support of the bidirectional gut-brain axis and its effects on the immune system, could lead to treatments involving the microbiome for inflammatory disorders.

The Inflammatory Model of Mood and Anxiety Disorders

Data supporting the theory that the gut microbiome affects different systems within various organism provides further evidence for the emerging theory of depression as an infectious or inflammatory disease. Canli proposes that in response to previous animal studies observing the effects of gut microbiota on behavior and inflammation (Bravo et al., 2011; Schmidt et al., 2014; Sudo et al., 2004), as well as examinations of inflammatory markers in regards to mood disorders (Dowlati et al.,

2010; Howren, Lamkin & Suls, 2009), depression should be reconceptualized as an inflammatory disease (2014).

Two recent meta-analyses (Dowlati et al., 2010; Howren et al., 2009), along with others (Maes et al., 1995; Mikova, Yakimova, Bosmans, Kenis & Maes, 2001), observed increased levels of specific immune cells in depressed participants when compared to participants without symptoms of depression. Dowlati et al. distinctly concluded that tumor necrosis factor alpha (TNF)-alpha, a regulatory cytokine and one of the inflammatory cells it regulates, interleukin-6 (IL-6), were found in increased levels in the blood of depressed participants when compared to non-depressed individuals (2010). In another meta-analysis by Howren et al., IL-6 was also increased in depressed participants as well as IL-1 and C-reactive protein (CRP), other immune cells whose production are triggered by inflammation (2009). CRP has also been found independently to be associated with higher levels of depression when plasma levels of CRP were observed in a cohort of 6,149 men with varying levels of depression (Danner, Kasl, Abramson & Vaccariano, 2003).

Desbonnet et al., conducted a study using mice models that provides a possible explanation of why inflammation is so closely associated with depression (2009). They found that when proinflammatory cytokines in mice, specifically IFN-gamma and TNF-alpha, decrease due to treatment with a single strain probiotic it correlated with an increase in tryptophan levels which is the main precursor to 5-HT (Desbonnet et al., 2009). These data show that inflammation is correlated with mood disorders, which indicates that these disorders may be influenced by more than neurotransmitter imbalance. This theory could aid in the development of

different treatments that target inflammation and the immune system and possibly improve treatment outcome for these types of disorders.

Probiotics

Probiotics are beneficial bacteria that live in the gut and positively affect organismal functioning via a mutualistic relationship. Not only have probiotics been more commonly added to food such as yogurt and kombucha naturally, but they have also been given as a supplement in numerous studies to either observe the results of altering the microbiome, or to test its possible therapeutic effects on a range of outcome variables involving mental and GI health. Probiotics were originally found to improve symptoms of intestinal discomfort, inflammation and motility (Kunze et al., 2009; O'Mahony et al. 2005), specifically with a focus on irritable bowel syndrome (IBS) (Hoveyda et al., 2009), but were later expanded to observe their effects on inflammation (O'Mahony et al., 2005), emotional reactivity and behavior (Bravo et al., 2011; Tillisch et al., 2013), mood and depression (Benton, Williams & Brown, 2014; Desbonnet et al., 2008; Messaoudi et al., 2010) and anxiety (Bercik et al., 2011; Bravo et al., 2011; Messaoudi et al., 2010). Although the mechanisms by which the microbes affect these factors is still underdeveloped, it has been proposed that metabolites such as short-chain fatty acids and neurotransmitters can be produced by the microbes and then alter the nervous, immune and endocrine systems of the host (Cryan and Dinan 2012).

O'Mahony et al. observed participants with IBS and found that a probiotic supplement, specifically *Bifidobacterium infantis* 35624, led to not only improved symptoms of IBS but also decreased plasma levels of the proinflammatory cytokines

IL-10 and IL-12 when compared to the placebo group (2005). They believe that the intestinal immune system of patients that experience IBS symptoms are not well able to down-regulate proinflammatory responses to antigens leading to more chronic inflammation and pain (O'Mahony et al., 2005). *Bifidobacterium* bacteria has also been found in lower levels in the feces of infants with atopy, an excessive inflammatory response, when compared to healthy infants (Björkstén et al., 2001; Kalliomaki et al. 2001), which may provide a possible explanation for the success found for decreasing inflammation with probiotics of this genera.

Researchers investigated the effects of probiotics, often of the *Bifidobacterium* and *Lactobacillus* genera in animals, by observing the brain and its composition via molecular techniques such as in Situ Hybridization to observe protein and receptor expression (Bravo et al., 2011) or imaging techniques such as fMRI (Tillisch et al., 2013). Tillisch et al. used an emotional responsivity task during fMRI to examine alterations in the activity of brain regions in response to ingestion of either a fermented milk product with probiotic (FMPP) containing *Bifidobacterium animalis* subsp *Lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, *and Lactococcus lactis* subsp *Lactis*, a non-fermented milk product, or nothing for four weeks (2013). fMRI data revealed that probiotic supplementation led to a decrease in blood oxygenation level-dependent (BOLD) activity in response to a negative emotional task in brain regions such as the insula, somatosensory cortex, and the prefrontal and frontal cortices as well as connectivity changes, specifically in the periaqueductal gray (PAG) which is known to play a role in response to stimuli (Tillisch et al., 2013).

Bravo et al. formulated an experimental procedure to test the effects of a lactic acid bacteria, *Lactobacillus rhamnosus*, on behavior and GABA receptor expression in healthy mice (2011). Differences were found in the behavioral task results between the two groups of animals with the probiotic fed mice exhibiting less anxious behaviors than the control mice. They found increased $GABA_{B1b}$ mRNA expression in cortical regions and decreased expression in areas of the brain associated with fear and anxiety (amygdala), memory (hippocampus) and mood (locus coeruleus). There was also a reduced expression of $GABA_{A\alpha2}$ mRNA in the prefrontal cortex and amygdala, but increased expression in the hippocampus (Bravo et al. 2011). These results were also only observed if the mice had an intact vagus nerve, therefore providing more insight into GABA and the vagus nerve as a possible mechanism as to how the bacteria affects the nervous system. Another bacterial species of the same genus, *Lactobacillus reuteri*, was found to increase enteric neuron excitability and ion channel activity, which subsequently affected motility of the intestines (Kunze et al., 2009). Since abnormal movement of the intestines causes common GI symptoms such as cramping, diarrhea, and constipation, the probiotic supplementation could improve these symptoms by properly regulating the enteric nervous system.

Data supporting the theory that the gut microbiome can cause alterations in the nervous and immune systems and subsequent disorders exist and have been discussed as a paradigm shift in Neuroscience; however, the majority of these studies are conducted using animals (Bravo et al., 2011; Kunze et al., 2009; Neufeld, Kang, Bienenstock & Foster, 2010; O'Mahony et al., 2005). One of the overall goals of

this research project is to better understand the bidirectional pathways between the gut, the brain and the immune system and the consequences of probiotics consumptions in young adults not recruited for gastrointestinal dysfunction or psychopathology. Prior to future treatment developments, many more studies involving human participants must provide support to preexisting data from animal studies and the small amount of preexisting clinical studies (Benton et al., 2006; Leyer et al., 2009; Tillisch et al., 2013).

This study aims to examine acute probiotic supplementation on depression, anxiety and GI symptoms in young adult participants. The effects of probiotics commonly used to positively alter the gut microbiota composition (Bercik et al., 2011; Bravo et al., 2011; Leyer et al., 2009; Messaoudi et al., 2010), on inflammation and mood and anxiety symptoms will be examined. Observations of the symptoms of depression, anxiety, and GI discomfort will be documented using self-report measures at baseline and post-treatment. Supplementation with VSL #3 probiotic blend, in comparison to a matched placebo, should lead to a larger decrease in symptoms of gastrointestinal functioning, anxiety, and depression.

Methods

Participants

Participants (N=100) were recruited from Allegheny College in Meadville, PA using the SONA online recruitment system as well as via group organizations on the Allegheny College campus. Participants signed up for a time slot via SONA to come into the laboratory. Once participants arrived at the laboratory for their appointment, they were given an informed consent form followed by packet of

questionnaires including the Center for Epidemiological Studies Scales of Depression (CES-D;Radloff, 1977), the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg & Jacobs, 1983), and the Gastrointestinal Symptom Rating Questionnaire (GSRQ;Hutchings et al., 2015). A final questionnaire assessed demographic data such as age, sex, race, relevant medical history, and antibiotic use.

Questionnaires

The Center for Epidemiological Studies Scales of Depression (CESD) (Appendix B) consisted of a 20-item questionnaire that assessed the level of depressive symptoms that participants had in the previous week (Radloff, 1977). Scores of 16 or greater, with a 60 being the highest score possible, were indicative of depression (Radloff, 1977; Schulberg et al., 1985), and scores 27 or greater indicated moderate to severe depression (Schulberg et al., 1985). Test-retest correlations ranged from .45 to .70 with the highest correlations occurring at shorter intervals (Radloff, 1977). The moderate correlations could be explained by the fact that this test was meant to assess short term symptoms of depression and mood which are expected to vary over time. The Cronbach's alpha was found to be between .84 and .90 and the split-halves r is between .76 and .85, both indicating high reliability (Radloff, 1977). At the second appointment, participants were asked to consider their mood for the previous two weeks.

The State-Trait Anxiety Inventory (STAI) (Appendix C) contained a section of 20 questions used to assess state anxiety and another section with 20 more questions to assess trait anxiety. All 40-questions used a 4 point Likert scale to

measure anxious symptoms (Spielberger et al., 1983). Section scores above 40 when measured out of the 80 possible points were indicative of clinical anxiety (Knight, Wall-Manning, & Spears, 1983). This measure was been tested for reliability and the total score was found to have a Cronbach's alpha of 0.86 and the test-retest correlation coefficients were highly significant (Quek, Low, Razack, Loh & Chua, 2004). At the second appointment participants were asked to consider their mood for the previous two weeks.

The Gastrointestinal Symptom Rating Questionnaire (GSRQ) (Appendix D) (Hutchings et al., 2015) was also used. This was a 30-item questionnaire that contained four different categories to assess the presence of various GI symptoms. The subscales assessed upper GI symptoms, lower GI symptoms, wind-related GI symptoms, and defecation-related symptoms. Each question could be given a response of “not at all”, “once a week”, “two or three times a week”, “most days”, or “every day” that suggested the presence and the severity of symptoms. The Cronbach's alpha for each subscale ranged from 0.70 to 0.85 and the intraclass correlations of each subscale, how closely the test and retest scores were related, were between 0.71 and 0.77 (Hutchings et al., 2015) indicating validity and reliability. At the second appointment participants were asked to consider their mood for the previous two weeks.

The Demographic and Early Life Exposure Inventory (Appendix E) was a combination of basic demographic questions that was used to describe the population as well as questions regarding the environment participants were exposed to as children. Participants were asked to provide their age, sex, ethnicity

and class year in the demographic portion. They were also asked questions regarding their birth, cigarette use, daycare attendance, siblings, pets, and antibiotic and antimicrobial use.

Probiotic: VSL#3

VSL #3 is a probiotic blend manufactured by Sigma-Tau Healthsciences and USA, Inc. Each capsule contained 112.5 CFUs of 8 different strains of live, freeze-dried bacteria. The species contained in this blend were *Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei,* and *Lactobacillus delbrueckii subsp. bulgaricus.*

Participants were asked to take two of these capsules or an indistinguishable placebo, starting the next day, at the same time of day, every day for two weeks. We suggested taking these pills right before they went to bed, or at a time of day when they would always remember to take them. We also asked participants that missed a dose and did not remember by the following day, to leave the capsules in the container. Participants were asked to return with the capsule container at their next lab session two weeks later. We also included these instructions on the back of the capsule container. When participants returned to the lab for their second session, their capsule containers were collected and the number of pills left in the container were recorded as "pills missed". A total of four participants missed six or more capsules during the two weeks, and were considered not compliant with procedure. Two of these participants were in the placebo group and two were in the probiotic group (Figure 1).

Procedure

Before participants began this double-blind and placebo-controlled study using random assignment they signed an informed consent form (Appendix A). Placebos and the probiotics were similar in appearance and were placed separately in identical boxes, with each box labeled with a letter containing a two-week supply. Participants were assigned to a letter using an online random number generator following a 60:40 allocation of probiotic to placebo. Before beginning the lab session, participants were asked if they had taken antibiotics in the past month or probiotics in the past six months; an affirmative response to either of these questions resulted in exclusion from the study. Participants then completed the CES-D, STAI, and GSRQ. All questionnaires were kept separate from the informed consent form, and were labeled with only a SONA identification number to ensure confidentiality. Before leaving the lab they were also asked to complete a demographics questionnaire.

Participants then received a 2-week supply of either the placebo or the probiotic and were informed to take two pills daily until all of the pills were gone. They also scheduled a follow-up meeting for 2-weeks later and signed up for daily text or email reminders. When they returned to the lab at their assigned time, they were asked to complete post-test measures. Participants completed the Gastrointestinal Symptom Rating Questionnaire (GSRQ) (Hutchings et al., 2015) as well as the CES-D (Radloff, 1977) and the STAI (Spielberger et al., 1983) again. Participants were thanked for their participation, and received a debriefing form (Appendix F) before leaving the lab.

Statistical Analyses

A mixed model ANOVA was used to test the hypotheses that there would be no significant group differences on any measure at baseline but that symptoms of depression, anxiety and GI symptoms would be lower at Time 2 in the probiotic group, and this effect would not be seen in the placebo group. There were two independent variables (time {baseline and Time 2} and treatment {placebo and probiotic}) both with two levels each and three dependent variables (CESD, STAI, GSRQ). A significant time x treatment interaction for each of the dependent variables would lend support for the study hypothesis. Main effects for time and treatment were not expected.

Results

One-hundred participants completed the baseline appointment, and data was incomplete for 8 participants and complete for 92 participants (Figure 1). Participant means and standard deviations for demographic, mood, anxiety, and GI health measures are reported in Table 1. To examine correlations between study variables independent of time, Pearson's correlation coefficients were calculated. Significant, positive correlations, were found between all variables within Time 1 (Table 2a) and Time 2 (Table 2b).

When variables were examined for normality using the Shapiro-Wilk test, only STAI scores at Time 1 were normally distributed (p=0.68, p=.118). Levene's test of equality of error variances was not significant for any of the dependent variables, demonstrating that the assumption of homogeneity of variance was met. To test the first hypothesis that acute supplementation with VSL#3 would decrease

depressive symptoms on the CESD, a mixed between/within ANOVA was run using a square root transformation for CESD data to account for normality. There was a main effect of time for the CESD total score, $F(1,91)= 21.94$, $p < .001$, showing that depression scores decreased from Time 1 to Time 2 collapsed across groups. There was no main effect of treatment $F(1,91)= .000$, $p=.988$, and no interaction between time and treatment $F(1,91)= 2.40$, $p=.125$, therefore not supporting the hypothesis that a two week probiotic supplementation would decrease depressive symptoms when compared to a placebo.

A mixed between/within ANOVA was run to test hypotheses about the effect of probiotic supplementation on anxiety and GI symptoms. Trait and state anxiety data at time two were unable to be normalized with square root, logarithmic, and natural logarithmic transformations, therefore the alpha-value of this ANOVA was set to 0.01 to reduce the risk of Type II error. There was no main effect of treatment on state anxiety $F(1,91)=.219$, $p=.641$, or trait anxiety $F(1,91)=.091$, $p=.764$. However, there was a main effect of time for trait anxiety $F(1,91)=16.517$, $p<.001$, showing that the trait anxiety score decreased from Time 1 to Time 2 collapsed across groups. This effect was not observed for state anxiety $F(1,91)=3.116$, $p=.081$. The interaction between time and treatment was not significant for state anxiety $F(1,91)=.470$, $p=.495$ or for trait anxiety $F=(1,91)=2.707$, $p=.103$, demonstrating no support for the hypothesis that probiotic supplementation would decrease anxiety when compared to the placebo.

Lastly, a mixed between/within ANOVA was run using logarithmic transformed variables for GSRQ data at Time 1 and a square root transformation for

Time 2 data. There was no main effect of treatment $F(1,90)=2.834$, $p=.096$, nor was there a time x treatment interaction $F(1,90)=2.177$, $p=.144$ for GI symptoms. There was a main effect of time $F(1,90)= 437.12$, $p < .001$. The above findings demonstrate that acute probiotic treatment does not reduce symptoms of depression, anxiety or GI symptoms. The above analyses were rerun with subsamples of the population to determine if individuals who scored above the cutoff for clinical depression (CESD >16) or anxiety (STAI >40), responded differently to the treatment. The results failed to show any significant time x treatment interactions.

Discussion

Daily consumption of a multistrain and multispecies probiotic for two-weeks failed to reduce self-reported depression, anxiety or GI symptoms in young adults (Table 1). There was no significant interaction between baseline versus Time 2 (time) and probiotic versus placebo (treatment) for scores on the CESD, STAI, and the GSRQ. These results do not support the original hypotheses that were formed based on preclinical studies where beneficial effects of probiotics on mood, behavior and physiological parameters have been noted (Bercik et al., 2011; Bravo et al., 2011; Desbonnet et al., 2008; Messaoudi et al., 2010; Tillisch et al.; 2013). It is possible that these results are explained by an absence of various pieces of this pathway/axis that allows the gut microbiota to communicate and alter the nervous system or an insensitivity of the measures that were used in the present study.

Part of the gut-brain axis theory originated from the findings that negative GI symptoms such as bloating, gas, constipation, and diarrhea, depression, and anxiety

are highly comorbid with each other (Lee et al., 2015; Whitehead et al. 2002). Our data were consistent with these findings. Significant positive correlations between CESD, STAI and GSRQ scores were noted in the current study (Table 2). This supports a growing body of literature linking central nervous and GI functioning with mental illness. Many possible mechanisms have been proposed as to how gut microbes, and supplemental probiotics, may affect depression and anxiety. Some researchers have proposed that the metabolites, that are sometimes neurotransmitters, produced by microbes interact with the nervous system (Bravo et al., 2011; Dinan and Cryan, 2012; Mayer, 2011). Other theories suggest that the gut-brain axis is modulated by the microbiota's effect on the immune system and inflammation (Canali, 2014; Dowlati et al., 2012; Ivanov and Littman, 2011; Olszak et al., 2012; O'Mahony et al., 2005).

A current challenge in this field of research is to determine if and how these mechanisms, largely examined in detail in animal models, translate to human physiology. When Kelly et al. measured stress-related behaviors, inflammatory response, cognitive performance, and brain activity patterns in healthy males in response to supplementation with *Lactobacillus rhamnous* (JB-1) they found that *L. rhamnous* (JB-1) did not decrease stress or inflammation or increase cognition in healthy males (2016). These findings were different from a preclinical animal study where the same probiotic strain altered GABA transmission and therefore behavior in mice (Bravo et al. 2011). This led Kelly et al. to conclude that before animal data supporting the positive effects of probiotics may be translated to humans, more must be known about the mechanisms at play (2016).

There has been however, data from human participants supporting the decrease in inflammation (O'Mahony et al., 2005), depression (Benton et al., 2006; Messaoudi et al., 2010), anxiety (Messaoudi et al., 2010), and emotional reactivity (Tillisch et al., 2013) in response to probiotic supplementation. There are many possible reasons for the dissonance between the previous mentioned studies and the present study. The strain of probiotic being used, the duration of the supplementation, the sample of participants, and the measurement is often inconsistent between various studies (Benton et al., 2006; Kelly et al., 2016; O'Mahony et al., 2005; Messaoudi et al., 2010; Tillisch et al., 2013). The current study specifically observed the effects of two-weeks of supplementation, which is shorter than many probiotic trials (Leyer et al., 2009; Messaoudi et al., 2010; Tillisch et al., 2013). For example, Leyer et al. treated children twice daily for 6 months (2009), Tillisch et al. provided probiotic supplementation for 4 weeks (2013), and Messaoudi et al. administered probiotics for 30 days (2010). Evidence suggests that microbes play an integral role in the development of the immune system at a young age (Rook et al., 2013; Penders et al., 2014), which subsequently affects inflammation and inflammatory disorders (Björkstén. 2004; Riedler et al., 2001) including GI disorders and depression. The present procedure may not have allowed enough time for the probiotic to colonize in the gut and alter the relative microbe compositions. The timing of colonization may be crucial if inflammation and the immune system is involved the pathway linking gut microbes and the nervous system (Rook et al., 2013; Penders et al., 2014).

It is unclear if lack of support for the original hypotheses in the current study is due to the fact that acute probiotic supplementation did not change the composition, if the composition did not affect mood, anxiety, and GI symptoms, or if it is due to extraneous variables or research limitations. Although the sample size was fairly large, it was limited to young adults attending college. The current study did not collect physiological measures such as inflammatory markers or fecal composition, linked to the gut-brain axis. Further, the present study did not have a rigorous measure of compliance. Measurements of proinflammatory cytokines such as IL-6, IL-10, IL-12, and CRP (O'Mahony et al., 2005) could provide information about the possible interplay between probiotics and the immune system and the inflammatory model of depression (Canli, 2014). If the microbiota composition of participants could have been analyzed via RNA sequencing of fecal samples (Kajander et al., 2007; Tannock et al., 2000; Tillisch et al., 2013; Wu et al., 2011) before and after treatment, it could have provided insight into the change the probiotic may have caused in the individual's microbiome. Although Tillisch et al. observed changes in emotional-responsivity in response to probiotic supplementation, they did not observe a significant difference in microbiota composition between groups after the intervention (2013). However, Kajander et al., observed an increase in microbial stability in the probiotic treated individuals and a decrease in stability in those given a placebo, with a significant difference between the two treatment groups (2007). This dissonance contributes to the need for further physiological measurements used in probiotic research with human subjects.

Different microbe compositions could also affect diverse organisms and individuals differently. If there exists a "healthy microbiome", it remains poorly understood what it is comprised of. It is possible that the implementation of multispecies and multistrain probiotics is not an efficient way to alter the microbe in the desired manner. Collins et al. gaveged mice with feces, and therefore full microbiomes from other mice, observed increased anxious behavior in normal mice that were gavaged with the feces of anxious mice. Probiotic studies introduced only a few different strains of bacteria into the gut (Bravo et al., 2011; Bercik et al., 2011; Messaoudi et al., 2010), whereas Collins et al. introduced an entire "anxious" microbiome that has a many different species that interact with each other in their relative compositions (2013) . It is also possible that human studies don't always correlate with the findings from animal studies due to psychological factors such as the Hawthorne effect. The present study saw a main effect of time with depression, which could be explained by the fact that participants knew their scores were being compared after a treatment. Future researchers may have to ask the question if complex human physiology and development can be altered dramatically enough with supplementation later in life to improve health and well-being.

Since the translation between animal studies and human studies is uncertain, more studies should be performed similarly to the current study, but with improvements. The examination of single probiotic strain, instead of multistrain, may be beneficial in determining which microbes are able to alter composition or release specific metabolites that will better affect the GI, nervous and immune systems. It may also be beneficial to observe specific diets that could affect the

microbiome and therefore how it interacts with the nervous and immune systems (Walker et al., 2011; Wu et al., 2011). The current study used one self-report questionnaire for each dependent variable, and including other measures as well, whether it be more self-report measures or physiological measures such as inflammatory markers or fecal samples may provide further insight into the mechanisms involved in the gut-brain axis in the human body.

Microbiome research has combined multiple different disciplines within the scientific community and has yielded convincing results in support of the existence of a gut-brain axis (Bercik et al., 2011; Bravo et al., 2011; Kunze et al., 2009; Leyer et al., 2009; Messaoudi et al., 2010; Tillisch et al., 2013). However, the complex mechanisms involved in this axis have made it difficult to understand the pathway and how animal research findings translate to humans. The current study provides insight into the effects of acute supplementation of probiotics and the possible gap between animal and human probiotic research. If probiotics, or other alterations of the gut microbiome, could provide relief for depression, anxiety, and comorbid diseases it could improve the quality of life for many individuals without the need for prescription medication. These inflammatory disorders could also be prevented by lifestyle changes early in life. Future research should be conducted to uncover more about the mechanisms connecting the gut microbiome and nervous and immune system in humans, in order to make further advances in alternative treatments for comorbid mood, anxiety and GI disorders.

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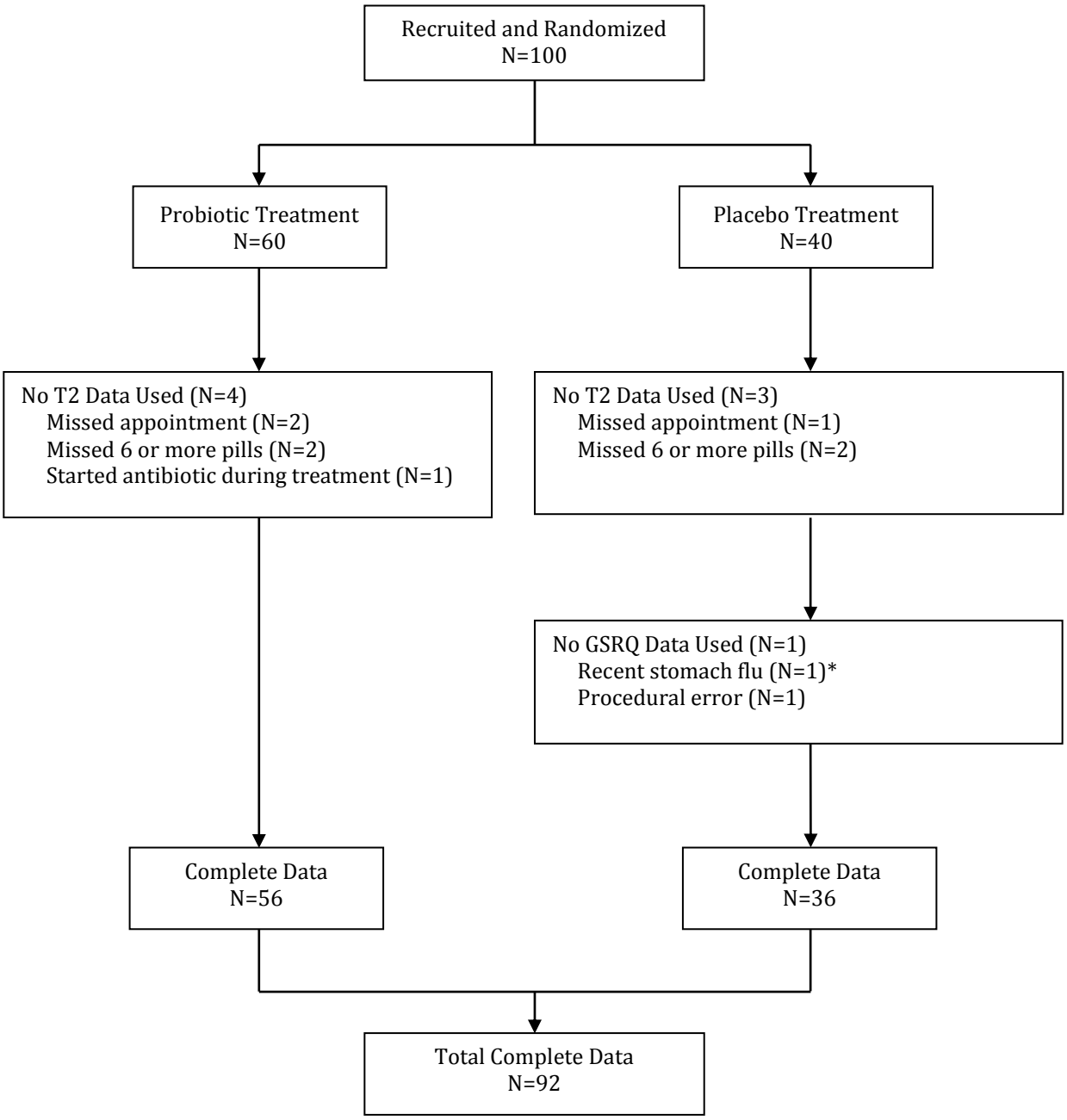


Figure 1. Consort Diagram
*This indicates a participant that was already excluded for missing more than 6 pills, therefore was not included in the total missing data for a second time.

Table 1.

Demographics, mood, anxiety, and gastrointestinal symptom data

	Total Sample		Probiotic		Placebo	
	T_1	T_2	T_1	T_2	T_1	T_2
Age	20.01[1.28]	-	20.05[1.31]	-	19.95[1.24]	-
Sex[1]	35(35)	-	20(33)	-	15(37.5)	-
Race[2]	81(81)	-	50(83.3)	-	31(77.5)	-
CESD	15.83[9.38]	12.62[8.22]	15.02[9.20]	13.09[8.53]	16.11[9.71]	11.92[7.78]
STAI State	39.61[10.81]	37.46[11.35]	39.29[11.29]	38.14[11.70]	39.03[10.17]	36.43[10.88]
STAI Trait	42.64[10.87]	39.46[10.94]	40.25[11.97]	42.20[11.52]	38.27[9.19]	42.86[10.04]
GSRQ						
Total	13.81[9.98]	12.87[8.95]	12.77[9.39]	11.63[8.15]	15.92[11.19]	14.81[9.86]
SS1	1.03[1.43]	0.78[1.23]	0.84[1.32]	0.73[1.26]	1.33[1.57]	0.86[1.20]
SS2	2.23[2.40]	1.48[1.65]	2.27[2.50]	1.50[1.76]	2.17[2.27]	1.44[1.50]
SS3	3.73[3.07]	3.97[3.12]	3.09[2.37]	3.39[2.84]	4.72[3.75]	4.86[3.36]
SS4	2.00[2.20]	1.76[1.95]	2.11[2.21]	1.52[1.60]	1.83[2.21]	2.14[2.37]
SS5	3.20[3.94]	3.27[3.82]	2.68[3.93]	2.91[3.48]	4.00[3.88]	3.83[4.28]

Note: Values are presented as mean[SD] or number(percentage) when appropriate. T_1 represents time one, and T_2 represents time two. [1]sex is expressed as percent male. [2]race is expressed as percent Caucasian. SS1 represents Subscale 1 – Heartburn. SS2 represents Subscale 2 – Reflux, Nausea, and Vomiting. SS3 represents Subscale 3 – Wind. SS4 represents Subscale 4 – Eating and Swallowing. SS5 represents Subscale 5 – Bowel Movements.

Table 2a.

Correlations between mood, anxiety, and gastrointestinal symptom scores at time 1

Variables	1	2	3	4
1. CESD	-			
2. STAI State	.561*	-		
3. STAI Trait	.705*	.731*	-	
4. GSRQ total	.444*	.322*	.366*	-

Note: *$p < 0.01$.

Table 2b.

Correlations between mood, anxiety, and gastrointestinal symptom scores at time 2

Variables	1	2	3	4
1. CESD	-			
2. STAI State	.717**	-		
3. STAI Trait	.760**	.804**	-	
4. GSRQ total	.333**	.215*	.164	-

Note: **$p < 0.01$, * $p < 0.05$.

Appendix A

Consent to Participate in a Senior Comprehensive Research Project

Participation in this study is completely voluntary and can be withdrawn at any time without consequences. This study aims to measure the relationship between stress and the use of a common, over-the-counter probiotic via questionnaires and physiological measurements after a brief laboratory task. Your participation in the study will help the researcher and the scientific community potentially gain understanding of the physiology of anxiety, depression, theory of mind, stress and stress-alleviation techniques through the contribution of this study to the literature.

This study will include two, sixty-minute lab sessions which will take place fourteen days apart. As part of the study, you will be asked to complete an extensive demographic questionnaire, in which you only need to answer to questions you feel comfortable with. You will be randomly assigned to take either a probiotic or a placebo every day for two weeks. Your assignment to either group will be double blind, meaning that neither you nor your experimenter will know which treatment group you are in until the end of the study. Neither the probiotic nor the placebo contain gluten, lactose, or soy ingredients. There is no more than minimal risk associated with participation in this study, but introduction of any new supplement into your diet may result in minor digestive irregularity.

Upon your completion of the study, you will be entered in a raffle to win a gift card to a local Meadville business if you choose to provide your email address at the end of each lab session. If selected, you will be contacted via email upon completion of the study.

Any information that you provide during your participation in this study will remain completely confidential. All data collected will be reported only as group means in tables and graphs, and your data set will be corresponded only to the participant number provided to you by the SONA system.

If you have any questions or concerns over the course of the study or if you experience any side effects, please contact Elizabeth Sever (severe@allegheny.edu), Francesca Carra (carraf@allegheny.edu), Nina Kikel (kikeln@allegheny.edu), Lora Waybright (waybrightl@allegheny.edu), faculty advisor Sarah Conklin (sconklin@allegheny.edu), or the Chair of the Institutional Review Board (IRB), Allison Connell-Pensky (aconnell@allegheny.edu).

By signing this form, I agree that I understand and consent to the information provided here. I also affirm that I am 18 years of age or older and am at liberty to provide consent in matters pertaining to my health and well-being.

Name: _____

Signature: _____

Date: _____

Appendix B

Center for Epidemiologic Studies Depression Scale (CES-D)

Below is a list of some of the ways you may have felt or behaved. Please indicate how often you've felt this way during the **past week**. Respond to all items.

Place a check mark (✓) in the appropriate column. **During the past week…**	**Rarely or none of the time (less than 1 day)**	**Some or a little of the time (1-2 days)**	**Occasionally or a moderate amount of time (3-4 days)**	**All of the time (5-7 days)**
1. I was bothered by things that usually don't bother me.				
2. I did not feel like eating; my appetite was poor.				
3. I felt that I could not shake off the blues even with help from my family.				
4. I felt that I was just as good as other people.				
5. I had trouble keeping my mind on what I was doing.				
6. I felt depressed.				
7. I felt that everything I did was an effort.				
8. I felt hopeful about the future.				
9. I thought my life had been a failure.				
10. I felt fearful.				

11. My sleep was restless.				
12. I was happy.				
13. I talked less than usual.				
14. I felt lonely.				
15. People were unfriendly.				
16. I enjoyed life.				
17. I had crying spells.				
18. I felt sad.				
19. I felt that people disliked me.				
20. I could not “get going”.				

Appendix C

State-Trait Anxiety Inventory

Directions:
A number of statements of which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.
1=NOT AT ALL, 2=SOMEWHAT, 3=MODERATELY SO, 4=VERY MUCH SO

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

Directions:
A number of statements of which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.
1=NOT AT ALL, 2=SOMEWHAT, 3=MODERATELY SO, 4=VERY MUCH SO

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

Appendix D

Gastrointestinal Symptom Rating Questionnaire (GSRQ)

Please answer the following questions by marking a cross in the box that best describes your symptoms. When answering the questions about the effect on your life, consider how these symptoms prevented you from doing your usual activities during the past 2 weeks.

0=Not at all 1=Once a week 2=Two or three times a week 3=Most days 4=Everyday

SECTION I - HEARTBURN

1. In the last two weeks, how often have you experienced heartburn (a burning sensation behind your breastbone)?
   0 1 2 3 4

2. In the last two weeks, how often have you had any discomfort in your upper abdomen (above your belly button and below your ribs)?
   0 1 2 3 4

If you have not had heartburn or upper abdomen discomfort, skip the next question and go straight to Section II over the page.

3. In the last two weeks, how much have the symptoms described in questions 1 and 2 prevented you from doing your usual activities?
   0 1 2 3 4

SECTION II - REFLUX, NAUSEA AND VOMITING

4. In the last two weeks, how often have you experienced bitter bile or acid reflux (from the stomach into the throat)?
   0 1 2 3 4

5. In the last two weeks, how often have you experienced a feeling of nausea or sickness without actually vomiting?
   0 1 2 3 4

6. In the last two weeks, how often have you retched or heaved without actually vomiting?
   0 1 2 3 4

7. In the last two weeks, how often have you actually vomited?
   0 1 2 3 4

If you have not vomited in the past 2 weeks, skip question 8 and go directly to question 9

8. If you have vomited in the last two weeks, have you seen any blood in the vomit?

0 1 2 3 4

9. In the last two weeks, how much have the symptoms described in question 4 to question 8 prevented you from doing your usual activities?

0 1 2 3 4

SECTION III - WIND

10. In the last two weeks, how often have you been bothered by a lot of belching (belching refers to the release of wind from the stomach via the mouth, often associated with feeling less bloated)?

0 1 2 3 4

11. In the last two weeks, how often have you been bothered by passing a lot of wind from your bowel?

0 1 2 3 4

12. In the last two weeks, how often have you experienced bloatedness, and or a feeling of trapped wind in your stomach?

0 1 2 3 4

13. In the last two weeks, how often have you experienced loud gurgling noises from your stomach?

0 1 2 3 4

14. In the last two weeks, how much have the symptoms described in question 10 to question 13 prevented you from doing your usual activities?

0 1 2 3 4

SECTION IV - EATING AND SWALLOWING

15. In the last two weeks, how often have you felt that your food sticks on the way down your gullet (through the chest into your stomach)?

0 1 2 3 4

16. In the last two weeks, how often have your eating habits been restricted because of your condition (examples might be having to eat more slowly, having to take smaller portions or having to eat different foods)?

0 1 2 3 4

17. In the last two weeks, have you had a lack of appetite?

0 1 2 3 4

18. In the last two weeks, how much have the symptoms described in question 15 to question 17 prevented you from doing your usual activities?

0 1 2 3 4

19. Have you noticed any change in weight over the last 3 months?

NO YES-losing weight YES- gaining weight

SECTION V - BOWEL MOVEMENTS

20. In the last two weeks, how often have you been bothered by too frequent emptying of your bowels?

0 1 2 3 4

21. In the last two weeks, how often have you been bothered by loose stools?

0 1 2 3 4

22. In the last two weeks, how often have you been bothered by hard stools?

0 1 2 3 4

23. In the last two weeks, how often have you been bothered by constipation (constipation means difficulty in emptying your bowels)?

0 1 2 3 4

24. In the last two weeks, how often have you had an urgent need to empty your bowels (this urgent need is often associated with a feeling that you are not in full control)?

0 1 2 3 4

25. In the last two weeks, how often have you had a feeling of not completely emptying your bowels?

0 1 2 3 4

26. In the last two weeks, have you had bleeding through your back passage (signs of bleeding include fresh blood, staining of toilet tissue, blood mixed with stools)?

0 1 2 3 4

27. In the last two weeks, how much have the symptoms described in question 20 to question 26 prevented you from doing your usual activities?

0 1 2 3 4

SECTION VI - OTHER QUESTIONS ABOUT SYMPTOMS DESCRIBED IN SECTIONS I TO V

28. Compared with two weeks ago, how would you rate these symptoms in general?

Much better Somewhat better About the same Somewhat worse Much worse

29. In the last two weeks, how often have these symptoms caused you difficulty in getting to sleep?

0 1 2 3 4

30. In the last two weeks, how often have these symptoms caused you to wake up?

0 1 2 3 4

Appendix E

Demographic and Early Life Exposure Inventory

Participant ID #:_____

Please circle all that apply and please do not skip any questions.

1) What is your age? 18 19 20 21 22 23

2) What is your sex? Male Female

3) Do you consider yourself (circle all that apply): White/Caucasian Native American Asian Hispanic/Latino Black/African American Pacific Islander Other: _____

4) What is your class year? FR SO JR SR Other:_____

5) Do you smoke cigarettes? Yes No

If yes to #5, how much do you smoke? < 1 pack a day >1 pack a day

6) Did you grow up in a rural area? Yes No

If yes to #6, did you live on a farm? Yes No

If yes to #6, did you spend a lot of time in wooded/forested areas? Yes No

7) Did you attend daycare before you were 5 years old? Yes No

8) If you answered yes to #7, what age did you begin daycare attendance? _____ N/A

9) How many siblings do you have? _____

10) Are you first born, a middle child, or last born in your family? First Second Third Fourth Fifth

Other:_____

11) Did you have animals your house during childhood? Yes No

If you answered yes to #12, what kind of pets did you have?

Circle all that apply and indicated how many.

Dogs_____ Cats_____ Reptiles_____ Birds_____ Other mammals_____ (Please list)_____ _____

12) Do you know how you were you born? (circle) Vaginal Caesarean Section Do not know

13) Do you know where were you born? Hospital Home Other:_____ Do not know

14) Do you know if you were you breastfed? Yes No Do not know

If yes, how long were you breastfed:_____

15) Have you used a broad-spectrum antibiotic (e.g. Penicillin, Amoxicillin, Ciprofloxacin, etc.) in the last month? Yes No

18) How many times, approximately, in the last year have you taken any kind of antibiotics?

0 1 2 3 4 5 6 More times than I can remember

19) In your lifetime, approximately how many times have you taken antibiotics?

0 1 2 3 4 5 6 More times than I can remember

20) Before the age of 5 were you ever hospitalized for any illness, injury or infection? Yes No

21) Have you ever received intravenous antibiotics? Yes No Unsure

22) Do you have allergies to any of the following? Please circle all that apply

dust mold pollen animal dander latex plants certain foods

23) Have you ever had allergy testing by a physician to confirm the above allergies? Yes No

24) Do you use antibacterial products such as antibacterial soap, hand sanitizer, etc.? Yes No

25) If you answered yes to #21, how often would you say you use hand sanitizer?

Everyday A few days a week Once a week Once a month

26) How often would you say you use other antibacterial products such as antibacterial wipes, antibacterial cleaning spray, etc.?

Everyday A few days a week Once a week Once a month Never

27) How would you like to be reminded to take your daily capsule? (Provide only one)

Email: _____

Text message (please provide phone number): _____

Appendix F

Debriefing Form

Purpose of Research:

Thank you for participating in this study. The purpose of this research study is to determine whether acute use of a probiotic will improve cardiovascular reactivity to mental stressors in healthy young adults, along with their effects on mood and theory of mind. Although research has been done to observe the effects of probiotic use on individuals with gastrointestinal diseases, very little research has been done to observe the effects probiotics have on healthy individuals. In addition, research using probiotics as a means to reduce cardiovascular reactivity to mental stress tasks and mood has only been seen using animal models, but not in humans. Due to the increasing popularity of probiotics, this study is relevant in order to show that probiotic use does actually have health benefits. We hypothesized that a two week use of a high-dose multi-strain probiotic mixture will reduce cardiovascular reactivity on a mental stress task and on a mood survey, and increase Theory of Mind ability in terms of mental state inferences and empathy as compared to a placebo-control. To be informed about which group you were randomly assigned to, or for the results of the completed study, please contact us using the phone number or email address found below.

Deception: We will not actually be evaluating your performance on the speech task. Minor deception was used in order to increase participant investment in the stressor and ensure a robust stress response. Please refrain from sharing the details of the stress task with anyone who might also participate in the study. Thank you.

Additional Sources of Information:

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Contact Information:

If you have any questions or would like additional information relating to this research, please contact gowithyourgut2017@gmail.com. You can also contact the research advisor, Dr. Sarah Conklin, at (814)-332-2392, or at sconklin@allegheny.edu. You may also contact Professor Allison Connell-Pensky, the chair of the IRB, at aconnell@allegheny.edu with any questions. If you have any health concerns please contact Winslow Health Center, (814)-332-4355, to make an appointment. If you have any additional concerns, please contact the Allegheny counselors for an appointment at (814)-332-3357, or in room 304 in Reis Hall.