EFFICACY OF PROBIOTICS IN IMPROVING MEMORY AND SYMPTOMS OF ANXIETY AND ADHD IN CHILDREN: A DOUBLE-BLIND STUDY

by

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Thesis submitted in partial fulfillment of the requirements for the Degree of Bachelor of Science with Honours in Psychology

Acadia University

March, 2016

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Acknowledgements

First of all, I would like to thank my supervisor, Dr. Susan Potter, for her continuous support and positive outlook throughout the entire process. Without her guidance, patience, and encouragement, this accomplishment would not have been possible. I can undoubtedly say this was a tremendous learning experience, and I am very grateful for all that I have learned, especially from Dr. Potter. Another warm thanks to my second reader, Dr. Michael Leiter, for his helpful feedback.

I would also like to thank everyone in the probiotics lab: Patrick Bazinet, Amanda Williams, Natasa Mitrovic, Billy Toulany, Dawn Armstrong, and Jeremie Shabani. Thank you for all of your hard work on the study and for creating the team atmosphere that encouraged my participation in the research process. Thank you to the other honours students. Their constant encouragement and support during the most stressful times was most appreciated.

Finally, I would like to say thank you to my friends and family for their encouragement throughout this entire process. Their ability to motivate me and provide emotional support proved invaluable to keep me on the track to completion.
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Abstract

In a world where there are limited treatments that do not have adverse side effects for children affected by anxiety and/or ADHD, the current study examined the therapeutic potential of probiotics for these conditions. The effects of probiotics on anxiety, ADHD, and memory were assessed in children aged 6 to 14 years. Participants (n=21) consumed probiotics or placebo for 4 weeks. Outcome measures included the Screen for Child Anxiety Related Emotional Disorders (Parent & Child versions), the Child Disruptive Behaviour Disorders Rating Scale (ADHD items only) and memory tests (visual & verbal). As hypothesized, visual memory improved in the probiotic, compared to the placebo, condition. Hyperactivity also improved, but in both groups. There were no other significant effects on any of the measures. However, many results were in the hypothesized direction. With a larger sample size, perhaps these results would reach significance.
Efficacy of Probiotics in Improving Memory and Symptoms of Anxiety and ADHD in Children: A Double-Blind Study

Researchers have long sought effective treatment options for mental disorders such as anxiety, depression, and attention deficit hyperactivity disorder (ADHD) that do not have harmful side effects. There are currently no medications approved by Health Canada for the treatment of anxiety in children (Health Canada, 2004), and the medications that are available for the treatment of ADHD often have undesirable side effects. Recently, probiotics, bacteria that have beneficial health effects, have been shown to have positive effects on anxiety and behavior in animals. However, there are limited studies examining the effects of probiotics on anxiety and behavior in humans, and none in children. The current study examined the effects of probiotics in children affected by anxiety and/or ADHD in a double-blind, placebo controlled clinical trial.

It has long been known that mental disorders originate in the brain, which is why brain processes have been targeted in the development of medication. Despite this fact, researchers have recently been considering other areas of physiological processing that may have an impact on mental health. In particular, the relationship between the gastrointestinal (GI) tract (where the microbiome resides) and the central nervous system (CNS) has been an area of heightened interest. A substantial number of animal studies have been done, predominantly modifying the gastrointestinal microbiome of rat subjects and observing improvements in behaviors related to mental disorders, such as anxiety (Bravo et al., 2011; Desbonnet, Garrett, Clearke, Bienenstock, & Dinan, 2008; Harding, Judah, & Grant, 2003). Human studies began to follow, to determine if the link between the gut and the brain is apparent in not only animals, but in human beings as well. In the
present day, mental health issues are becoming more recognized, and many people are turning toward alternative treatment options to avoid the potentially negative side effects of medications. New alternative treatments are beginning to target the gut microbiome.

**The Microbiome**

A substantial diversity of microbes occupies many surfaces throughout the human body, including the inside of the gut, known as the gut lumen (Fukuda & Ohno, 2014). The human gastrointestinal tract consists of over 100 trillion microbes, categorized into no less than 1,000 different species. The total number of gut microorganisms surpasses the approximately 60 trillion somatic cells within the human body substantially (Fukuda & Ohno, 2014). Many physiological processes can be affected by the gut microbiome including brain functioning and the communication between the gut and the brain (Cryan & Dinan, 2012). The gut microbiome also contribute to nutrient absorption, as well as maintaining the stability of the cells lining the GI tract. Furthermore, it plays an important role in the maturation of the immune system, defense against pathogens and disease, drug metabolism, and harvesting energy from food (Fukuda & Ohno, 2014).

The establishment of the gut microbiome is a postnatal occurrence. The development of the microbiome begins at birth, when vaginal delivery exposes the child to an elaborate microbiome from the mother. It only takes about one year for the infant to develop a mature and intricate adult-like microbiome (Cryan & Dinan, 2012). The method used to deliver babies has been shown to result in differences in their intestinal microbiome (Dominguez-Bello et al., 2010). In a study by Dominguez-Bello et al., nine women aged 21 to 33 gave birth either vaginally or via C-section, and the microbiota of the babies were analyzed. It was found that infants delivered vaginally acquired bacterial
communities that were very similar to their mother’s vaginal microbiota, whereas C-sections gave rise to bacterial communities typically found on the surface of the skin (Dominguez-Bello et al., 2010). Penders et al. (2006) concluded that there are several additional factors that contribute to the determination of the gut microbiome composition, including the method used to feed the child (i.e., breastfeeding, bottle feeding), gestational age, and the amount of antibiotics consumed by the child. Infants who were exclusively breastfed had the highest number of beneficial gut microbiota (i.e., Bifidobacteria); infants who were administered antibiotics within the first month of their life, as well as those who were born prematurely, had decreased numbers of beneficial bacteria (Penders et al., 2006).

The specific make-up of the gut microbiota may play a role in the development of some negative health issues. Researchers have suggested that without adequate amounts of certain bacterial groups, defense against foreign attack is difficult (Backhed et al., 2012) and may cause individuals to become susceptible to a collection of diseases (Fukuda & Ohno, 2014). Beneficial bacteria contribute to developing and strengthening the immune system. Decreased or absent amounts of these beneficial bacteria may contribute to inflammatory responses (Kaplan, Rucklidge, Romijn, & McLeod, 2015). These inflammatory responses may play a fundamental role in the development of a number of long-term inflammatory disorders, such as inflammatory bowel disease and rheumatoid arthritis, as well as certain mental disorders (specifically depression) which seem to be related to heightened inflammatory activation (Kaplan et al., 2015).

Despite the fact that the combination of bacteria that comprise the microbiota in every individual is unique, the size of the colonies and the locations throughout the
intestine are very much alike among healthy human beings (Carabotti, Scirocco, Maselli, & Severi, 2015) and among those with similar medical conditions. Willing and colleagues (2010) found that the microbial communities varied depending on the disease phenotype displayed. For example, comparable quantities and compositions of bacterial communities were consistent with irritable bowel disease phenotypes. There was a distinguishable difference in the microbiota of healthy individuals compared to individuals with these gastrointestinal diseases. Another example of this is seen in individuals with autism. Researchers have discovered that gastrointestinal disorders are common in persons with autism. More diversity of microbiota can be seen in those with severe autism compared to matched controls. This higher diversity may contain dangerous species within the phylum of Bacteroidetes and Firmicutes, which may be instrumental in affecting the severity of autistic symptoms (Finegold et al., 2010). Accordingly, it is imperative to understand the gut microbiota and their association with disorders in order to develop effective treatments (Fukuda & Ohno, 2014).

**Probiotics**

According to Sanders (2008), the internationally endorsed definition of probiotics is “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (p. 58). A bacterium that is considered to be probiotic should possess certain characteristics that differentiate it from the thousands of other strains of bacteria living in the gut. Probiotics should be incapable of causing disease and they should remain in the gut for long enough to exert their beneficial effects. The strain should be stable in gastric acid and bile and be hostile to pathogenic bacteria in order to be beneficial to the host. Finally, it is suggested that probiotics should be capable of
regulating the immune response (Di Giancamillo, Vitari, Bosi, Savoini, & Domeneghini, 2010).

It has been shown that only about 20-40% of consumed probiotics survive by the time they arrive at the lower GI tract because of the harsh stomach acid that produces a highly acidic environment (Bezkorovainy, 2001). In order for probiotics to successfully reach the part of the GI tract where they perform their beneficial effects, researchers have discovered that probiotics should be consumed at an optimal time relative to the time other food is ingested. Tompkins, Mainville, and Arcand (2011) found that probiotics were most likely to survive when taken with, or 30 minutes before a meal, as opposed to 30 minutes after a meal. The probiotics are able to pass through the stomach much more freely when there is minimal food present. Another method to protect probiotics in the gut involves microencapsulating them in a polymer matrix made from natural polysaccharides and proteins, which are not harmful to the host or the bacteria. This microencapsulation protects and prevents the probiotics from breaking down when travelling through the digestive system (Cook et al., 2012). The probiotics must reach the intestines and colon of the GI tract in order to be successful in exerting their benefits.

After probiotics are consumed and have persisted briefly in the gut lumen in adequate numbers, these bacteria may be capable of having a positive impact on gastrointestinal function because of their biochemical connection with different parts of the intestinal wall (Di Giancamillo et al., 2010). Probiotics often contain lactic acid-producing bacteria that are members of the genera *Lactobacillus* and *Bifidobacterium*. Many studies have documented the positive effects of these probiotic bacteria in providing relief of symptoms related to irritable-bowel syndrome (Zeng et al., 2008).
Probiotics are not only used to mitigate symptoms of gastrointestinal disorders. A recent study by Tillisch et al. (2013) found that consumption of probiotics could affect brain activity. Healthy women were given either a fermented milk product containing probiotic bacteria or a non-fermented milk product, or nothing. Functional magnetic resonance imaging (fMRI) was performed, measuring brain activity by identifying changes in blood flow. Significant changes in brain activity were observed in those who consumed the probiotics compared to the groups who had no probiotics. The probiotic bacteria seemed to specifically influence brain activity in areas that are responsible for the processing of emotion (e.g., insula) and sensation (e.g., thalamus) (Tillisch et al., 2013).

Benton, Williams, and Brown (2007) studied whether probiotics had an effect on general well being as measured by changes in mood and memory. Participants received either probiotics or a placebo for three weeks. Mood was measured using the Profile of Mood states (POM) questionnaire (Lorr & McNair, 1984) as a baseline, and was also evaluated daily using a scale in which the participants indicated which adjective applied to their mood on that particular day. This study demonstrated that the ingestion of a yoghurt drink containing probiotics enhanced the mood of individuals who were, at baseline, more depressed. However, no significant effects on memory were observed.

Probiotics have recently been shown in animal studies to have beneficial effects on memory. Davari, Talaei, Alaei, and Salami (2013) subjected rats to a Morris water maze task and compared diabetic rats’ functioning on the task with control rats. Probiotics were provided for 56 days and their consumption was associated with significant memory improvements in both groups of rats. In a similar study by Matthews and Jenks (2013), memory improvements were seen when the bacteria *Mycobacterium vaccae* was given to
healthy mice. The mice that consumed *Mycobacterium vaccae* were twice as fast at completing the maze task as the control mice that did not receive the supplement.

**Probiotics and Mental Health**

Anxiety disorders are one of the most prevalent mental disorders (APA, 2013). Anxiety disorders typically begin in childhood or adolescence and are often recurrent over the rest of the affected individuals’ lives (Kessler et al., 2009). Individuals with anxiety disorders experience constant, unreasonable worry and anxiety, with these feelings occurring more often than not and consequently affecting their everyday lives (Gorman, 2003). Comorbidity with other anxiety disorders and with other mental health disorders is very high (Kessler et al., 2009). There are many subtypes of Anxiety Disorders in the DSM-5, such as separation anxiety disorder, specific phobia, panic disorder, agoraphobia, social anxiety disorder, and generalized anxiety disorder (APA, 2013).

Much of what we know about the connection between probiotics and mental health stems from animal studies. In a study by Bravo et al. (2011), either probiotics containing *Lactobacillus rhamnosus* or a placebo were administered to healthy mice. The mice were subjected to a series of behavioral evaluations related to anxiety and depression-like behavior. It was found that probiotic treatment decreased the amount of anxiety and depression-like behaviors in the mice. Results also showed that probiotics generated changes in the GABAergic system (GABA is the main inhibitory neurotransmitter in the CNS, reducing neuronal excitability), the target system of many anxiolytic drugs acting on the CNS, within regions of the brain known to be responsible for regulating anxiety and depression-like behaviors (Bravo et al., 2011). Additionally, the amino acid tryptophan is a necessary precursor for the synthesis of the
neurotransmitter serotonin. This is important when considering brain chemistry and imbalances of serotonin associated with anxiety and depression (Pinel, 2013). Desbonnet et al. (2008) treated rats with *Bifidobacteria infantis* for 14 days and found a significant increase in tryptophan levels. The probiotics also diminished the pro-inflammatory immune response that Kaplan et al. (2015) had linked to mental disorders such as depression. Furthermore, a study by Arseneault-Breard (2012) demonstrated that a probiotic formula consisting of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 reversed the depression-like behavioral consequences of myocardial infarction in rats and restored intestinal barrier strength.

Research with animals has been very important in uncovering the effects of probiotics on psychological disorders, but further work was needed to apply this research to human subjects. Messaoudi et al. (2011) showed that *Lactobacillus helveticus* and *Bifidobacterium longum* reduced anxiety-like behavior in rats. However, this study also assessed the psychotropic effects of probiotics in humans. The adult volunteers were given either probiotics or placebo for 30 days, and symptoms of anxiety and depression were monitored using a variety of standardized measures. Those who took probiotics daily experienced beneficial psychological outcomes, further supporting the use of probiotics in reducing feelings of stress, anxiety and improving overall mood in humans (Messaoudi et al., 2011).

Studies of individuals with autism spectrum disorder (ASD) also suggest a positive link between anxiety and probiotics. Individuals with ASD commonly exhibit higher levels of intestinal permeability and imbalance of microbiota. Hsiao et al. (2013) treated individuals affected by ASD with *Bacteroides fragilis* and positive results were
observed, including improvements in ASD-associated problems in communicative, stereotypic, sensorimotor, and anxiety-related behaviors. Additionally, *Bacteroides fragilis* repaired gut permeability and adjusted the microbial configuration.

Not only do probiotics seem to have beneficial effects on anxiety and depression, but probiotics have also been shown to be beneficial in the treatment of ADHD. According to the DSM-5, ADHD is a pattern of behavior including symptoms such as “failure to pay close attention to details, difficulty organizing tasks and activities, excessive talking, fidgeting, or an inability to remain seated in appropriate situations” (APA, 2013). This disorder is believed to occur in about 5% of children (APA, 2013). ADHD is a disorder that originates in childhood, but may carry on into adulthood for some. Studies have shown that individuals diagnosed with ADHD differ from non-ADHD individuals in terms of microbial composition and bacterial diversity. For instance, Pärtty, Kalliomäki, Wacklin, Salminen, and Isolauri (2015) found that children with ADHD have fewer species of *Bifidobacterium* in their stool specimens than children without ADHD. Additionally, in a meta-analysis of memory function in children with ADHD, it was found that these individuals displayed deficits in memory compared to control subjects without the disorder (Martinussen, Hayden, Hogg-Johnson, & Tannock, 2005). For years the treatment of ADHD has involved stimulant pharmacotherapy and occasionally psychosocial interventions. It appears that stimulants are effective in the treatment of ADHD, but more than 30% of patients show an insufficient response or are unable to endure the stimulant treatment (Abbasi et al., 2011). This is the reason behind the need for alternative treatment options for this disorder.
A few studies exist in which the treatment of ADHD with probiotics has been examined. In a study by Harding and colleagues (2003), 20 children with ADHD ages 7-12 years old were examined. Ten children were prescribed Ritalin and the other ten were prescribed dietary supplements (consisting of a multiple vitamin, multiple mineral, phytonutrients, essential fatty acids and phospholipids, probiotics, and amino acids). An Intermediate Visual and Auditory/ Continuous Performance Test was administered and significant attention improvements were seen in both groups equally (Harding et al., 2003). Although the specific effects of probiotics were difficult to isolate from the other components of the supplement in this experiment, food supplements were just as effective as medication in these children. This suggests that there may be healthier alternatives to stimulant medication.

In a more recent study, mentioned previously, by Pärty and colleagues (2015), 75 newborn babies were allocated to receive either a probiotic supplement or a placebo for the initial six months of life. These children were then followed, and it was observed that children who went on to develop Asperger’s syndrome or ADHD had lower levels of *Bifidobacterium* in their fecal samples during their first six months of life. By the time they were 13 years old, 17% of the children in the placebo group had developed Asperger’s syndrome or ADHD; not one of the children in the probiotic condition had developed a psychological disorder. This suggests that probiotic consumption within the first six months of life may play a role in preventing the development of ADHD and Asperger’s syndrome.
Gut-brain Axis

Gut microbiota, along with probiotics, can change brain functioning and brain neurochemistry (Ait-Belgnaoui et al., 2012). The enteric nervous system is a branch of the peripheral nervous system that controls the gastrointestinal system; it has also been described as “the second brain” (Gershon, 1999). The enteric nervous system communicates with the central nervous system via a bidirectional communication system, which researchers have labeled the “gut-brain axis” (Saulnier et al., 2013). The gut-brain axis provides a mechanism for the brain’s emotional and cognitive processing centers to influence enteric nervous system functions and vice versa. These two systems include varying routes of communication, including the vagus nerve (the most direct route), the HPA axis (the neuroendocrine route), and the immune system, which involves substances released by immune cells that are involved in cell signaling (e.g., cytokines). Via these pathways, the enteric nervous system is able to relay sensory information to the CNS, and the CNS is able to have an impact on the function of the gastrointestinal tract.

There are also indications that the intestinal microbiota play a major role in gut-brain axis communication (Bravo et al., 2012). One of the essential impacts of the microbiota on the gut-brain axis comes from the regulation of the intestinal barrier, where the repair of stable tight-junctions and preservation of the intestinal barrier are very important to the function of the gastrointestinal system as a whole (Carabotti et al., 2015). The intestinal epithelial barrier regulates the absorption and exchange of materials between the lumen and the circulatory system and helps prevent toxic substances from passing through the barrier and harming the host. By allowing toxins into the bloodstream, an impaired intestinal barrier may have an indirect effect on the brain and
play a role in the inflammation seen in mental disorders (e.g., depression) (Fasano & Shea-Donohue, 2005). Stressful circumstances have been shown to cause the gut to become “leaky”, allowing these potentially harmful molecules to pass from the gut into the bloodstream (Saulnier et al., 2013; Cryan & Dinan, 2012). Additionally, microbiota can communicate with the gut-brain axis by regulating afferent sensory nerves (which travel to the CNS) that control gut motility (coordination of muscle activity in the gut) and pain awareness. Microbiota can also make chemicals that function as neurotransmitters (e.g., GABA, serotonin, melatonin, etc.). Finally, microbiota can create a functional form of catecholamines in the gut lumen, thereby affecting the functioning of the enteric nervous system (Carabotti et al., 2015).

The strong comorbidity between psychiatric conditions, such as anxiety, and gastrointestinal disorders, such as irritable bowel syndrome (IBS), further implicates the gut-brain axis in the pathophysiology of these conditions, making it an appealing focus for research into new treatment options (Cryan & Dinan, 2012). This relationship between gastrointestinal disorders and the brain was evident in a study comparing university students with IBS, to those who did not have the disorder (Gulewitsch, Enck, Schwille-Kiuntke, Weimer, & Schlarb, 2011). Gulewitsch et al. found that 40% of the group with IBS displayed symptoms of chronic anxiety and stress, while only 20% of the group who did not have IBS displayed these symptoms.

One route of communication between the gut and the brain is likely mediated through the vagus nerve. In a study previously mentioned, Bravo et al. (2011) analyzed whether the beneficial effect of the probiotic *L. rhamnosus* on behavioral symptoms of anxiety and depression in mice may be controlled by the vagus nerve. The researchers
discovered that among mice receiving the probiotic, the mice whose vagus nerve had been severed (vagotomy) did not display the anxiolytic (anxiety reducing) and antidepressant effects on behaviour that were seen in the mice with intact vagus nerves (Bravo et al., 2011). This supports the claim that the vagus nerve is an important communication route between the microbiota of the gut and the brain. Further evidence for the role of the vagus nerve in gut-brain communication was observed by Bested and colleagues (Bested, Logan, & Selhub, 2013). They induced weak gastrointestinal inflammation in animals and observed an increase in anxiety-like behavior. When this was done in animals that had received a vagotomy, the same anxiety-like behavior was not present.

**Relationship Between Stress and the Gut-Brain Axis**

It has been shown that the gut microbiota influence the HPA reaction to stress, and chronic HPA activation can influence the make-up of the gut microbiota. Possibly the most important components of the body involved in the relationship between stress and the gut-brain connection are the hypothalamus, pituitary, and adrenal glands that form the HPA axis. A stressful situation triggers a chain reaction in the HPA axis, beginning with the release of corticotropin-releasing factor (CRF) from the hypothalamus, followed by adrenocorticotropic hormone (ACTH) from the pituitary gland. This results in the release of cortisol from the adrenal glands (Carabotti et al., 2015; Cryan & Dinan, 2012). Cortisol (known as corticosterone in rats and mice) is a vital stress hormone that influences many organs of the body, especially the brain (Carabotti et al., 2015). Cortisol raises blood sugar levels, and controls fat, protein, and carbohydrate metabolism in response to stress. High cortisol levels in saliva have been associated with anxiety disorders (Vreeburg et al., 2010), whereas lower levels of cortisol have been associated
with ADHD (Ma et al., 2011). Thus, both disorders seem to be associated with impairment in the HPA axis.

Following perinatal microbial colonization, the gastrointestinal microbiome can have an enduring effect on the way the brain processes the type of sensory information that has the potential to activate an HPA axis response (Sudo et al., 2004). Maternal separation is considered an “early life stressor” that has been shown in mice to cause enduring heightened activity of the HPA axis and long-term effects on the gut microbiota. Similar negative effects of maternal separation on the stress response were found among rats, in a study by O’Mahony et al. (2009). In this study, rats experienced maternal separation for three hours a day, for 10 days following birth. Corticosterone levels were higher in the animals that were separated at birth, and an increased immune response was observed. Altered fecal microbiota were seen as well. The long-lasting effects of this early life stressor were clear in the animals’ immune responses, behaviors, and HPA axis functioning, leading O’Mahony and colleagues to conclude that early life stressors promote long-term alterations within the gut-brain axis.

**Probiotics and Cortisol**

Evidence that probiotics attenuate the stress response has been seen in the form of lowered cortisol levels following probiotic administration. This was first examined in animals. Gareau, Jury, MacQueen, Sherman, and Perdue (2007) investigated the impact of probiotics on the stress response in maternally separated rat pups. These stressed rat pups were compared with non-separated, non-stressed pups after the administration of probiotics (two strains of *Lactobacillus*) for 20 days. The consumption of probiotics led to the alleviation of gut functional irregularities, and decreased the high levels of
corticosterone in the maternally separated pups (Gareau et al., 2007), suggesting that probiotics can reduce the physiological effects of stress.

Similar beneficial effects of probiotics on the stress response were also observed when probiotics were tested with human subjects. Messaoudi et al. (2010) found that healthy human participants had significantly lower cortisol levels after 30 days of receiving *L. helveticus* R0052 and *B. longum* R0175, whereas a decrease in cortisol was not observed among the placebo controls. These results provide further evidence for the role of probiotics in the modulation of HPA axis activity.

**The Present Study**

The types of medications used to treat anxiety, such as selective serotonin reuptake inhibitors (SSRIs), can cause many adverse effects. These harmful side effects can include weight gain, nausea, gastrointestinal disturbances, sleep disturbances, and more severely, addiction (Ferguson, 2001). Health Canada does not recommend any SSRI medications for children under the age of 18 because of the harmful side effects and insufficient effectiveness (Health Canada, 2004). While anxiety has one of the highest rates of diagnosis, there are no safe pharmaceutical treatments for the disorder in children; thus, where access to psychological services is limited by finances or lack of availability, these children have very few treatment options. A safe and effective treatment is needed for children who suffer from anxiety.

In addition, stimulant medication for the treatment of ADHD (such as Ritalin™) can cause unfortunate side effects such as insomnia, headaches, and loss of appetite, and the medication may show no efficacy at all in some people (Ahmann et al., 1993). These side effects can be particularly harmful in children, which is why a different method of
treatment with fewer adverse effects would be extremely beneficial for children diagnosed with ADHD.

There is mounting evidence to suggest that the gut microbiota may play a role in anxiety and ADHD via the gut-brain axis. Moreover, animal and human studies have suggested that probiotics may have a positive impact on the gut-brain axis, and via various mechanisms, including effects on the HPA axis. Since probiotics are naturally occurring microorganisms noted for their beneficial effects and lack of pathogenicity, it is very unlikely that probiotics would cause any of the adverse side effects seen with anxiolytic and stimulant medications (Ishibashi & Yamazaki, 2001).

The current study is a double-blind, placebo-controlled clinical trial examining the effects of probiotics on memory and symptoms of anxiety and/or ADHD in children. The current study is part of an on-going, larger study that, in addition to the memory, ADHD, and anxiety outcome measures discussed in this thesis, includes the analysis of salivary cortisol levels, attention, gastrointestinal symptoms, and diet, which will be described in separate reports. In addition, the larger study, once complete, will include 100 participants; the current study was based on the first 21 participants to complete the first phase of the study (that is, four weeks of probiotic or placebo).

The probiotic formulation investigated in this study is Lallemand’s proprietary combination of *L. helveticus* R0052 and *B. longum* R0175. Past research investigating the effects of probiotics on memory and symptoms of anxiety and ADHD in humans is lacking in general, but even more specifically, in children. The aim of this study is to examine whether treatment with probiotics is effective in improving memory, and reducing symptoms of anxiety and ADHD in children. Previous research has not
considered the high comorbidity rate when dealing with ADHD and anxiety, and whether lowering the effects of one may moderate the other. This study will look specifically at children with symptoms of anxiety, ADHD, or both.

Based on previous research, three main hypotheses were considered for this study. First, it was hypothesized that improvements in symptom scores on measures of ADHD and anxiety symptoms would be seen among participants who consumed the probiotic but not among those who consumed the placebo. Second, it was hypothesized that a similar pattern would be seen on the memory tasks; that is, improvements in memory would be seen among participants who consumed the probiotic but not among those who consumed the placebo. Third, it was hypothesized that there would be a significant relation between improvements in memory and improvements in ADHD symptoms, and between improvements in memory and improvements in anxiety symptoms.

Method

Participants

Twenty-one children aged 6 to 14 years, with symptoms of anxiety and/or ADHD participated in this study. These were the first 21 participants to complete the first phase of the larger study before the end of February 2016. Recruitment of these participants was conducted across Nova Scotia, on the condition that the participants were able to meet with the researchers personally in Wolfville or in Halifax, NS. Participants met with the researchers at either the Acadia University Probiotics and Mental Health Lab, or at Roth & Associates Mental Health Clinic in Halifax. Parents or guardians of the participants were required to have access to a computer and the Internet to fill out questionnaires necessary for the study, and an e-mail address in order to keep in contact with the
researchers for the duration of the study. Prospective participants were excluded for any of the following reasons: currently taking probiotics or antibiotics; having dairy intolerance; having HIV/AIDS; currently undergoing chemotherapy; having Crohn’s disease, ulcerative colitis, or acute pancreatitis; having an immune-compromised condition (e.g., AIDS, cancer, lymphoma, undergoing long-term corticosteroid treatment); or having a soy or milk allergy. Participants were paid $50 for their participation upon completion of the study.

The sample was predominantly male (male = 17, female = 4), with a mean age of 9.8 years, and an age range of 6 to 15 years. The average grade level completed in school was grade five. Participants were divided into two groups: Probiotic (Male = 9, Female = 2) and Placebo (Male = 8, Female = 2). The children in the Probiotic condition had a mean age of 10.45 years, and their average grade level in school was 5.7. The children in the Placebo condition had a mean age of 9 years, and an average grade level of 4.4. In general, the parents of the participants were working middle-class citizens.

To examine mental health symptoms and problem behaviours among participants, and to identify any differences between groups on these variables, a parent or guardian of each participant completed the Child Behavioral Checklist (CBCL). Prior to analyses, T-scores for the Total Problems scales (Total score, Internalizing Problems and Externalizing Problems), the DSM-Oriented scales, and the Syndrome scales were examined. Mean T-scores for each subscale were computed, and were classified as “Normal” (T-score of < 60), “Borderline Clinical Range” (T-score of 60-63), and “Clinical Range” (T-score of > 64). A between-subjects MANOVA was performed with Condition (Probiotic or Placebo) as the independent variable, and mean T-scores on the
CBCL problem measures at baseline as the dependent variables. The Multivariate test was not significant, $F(3, 17) = .382, p = .915$, indicating that the probiotic and placebo groups had similar levels of emotional/behavioural problems overall, as measured by the CBCL.

In order to gather information about mental health symptoms among participants that could be used to tweak the eligibility criteria of the larger study, the means and standard deviations of the scores that were approaching significance (Externalizing Problems, Somatic Problems, ODD Problems, Withdrawn/ Depressed, and Aggressive Behavior) were examined. As shown in Table 1, it was found that the mean T-scores for all of the near-significant problem behaviors were higher in the Probiotic condition. Four scales were in the clinical range, and two scales were in the borderline clinical range in the Probiotic group, whereas in the Placebo condition, three scales were in the normal range, three scales were in the borderline clinical range, and none of the scales were in the clinical range. This indicates that participants in the Probiotic condition may have exhibited somewhat more severe Externalizing Problems, Somatic Problems, ODD Problems, Aggressive Behavior, and were more withdrawn/ depressed than participants in the Placebo condition at baseline, although none of these differences were statistically significant at $\alpha=.05$. On all other problem behaviors, the Probiotic and Placebo conditions were similar at baseline ($p > .15$).

**Materials**

The present study involves the first phase of a longer (13 week), multi-phase research study that includes a number of measures that are beyond the scope of this thesis. Only those materials and measures that are relevant to this thesis are described in detail; other measures will be mentioned briefly.
Table 1  
*CBCL Problem Behavior (From DSM-Oriented Scales and Syndrome Scales): Means and Standard Deviations for the Probiotic Condition (n=11) and the Placebo Condition (n=10)*

<table>
<thead>
<tr>
<th>Problem Behavior (T-scores)</th>
<th>Probiotic</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DSM-Oriented Scales</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internalizing</td>
<td>63.64 (8.39)</td>
<td>59.00 (9.12)</td>
</tr>
<tr>
<td>Externalizing</td>
<td>66.00* (9.78)</td>
<td>59.60* (6.47)</td>
</tr>
<tr>
<td>Total Problems</td>
<td>67.27 (7.71)</td>
<td>62.90 (6.89)</td>
</tr>
<tr>
<td>Affective Problems</td>
<td>63.27 (7.80)</td>
<td>60.30 (7.45)</td>
</tr>
<tr>
<td>Anxiety Problems</td>
<td>63.00 (10.75)</td>
<td>60.70 (9.94)</td>
</tr>
<tr>
<td>Somatic Problems</td>
<td>62.18* (7.48)</td>
<td>57.00* (7.16)</td>
</tr>
<tr>
<td>ADHD Problems</td>
<td>66.73 (7.04)</td>
<td>67.60 (6.13)</td>
</tr>
<tr>
<td>ODD Problems</td>
<td>66.45* (9.73)</td>
<td>60.30* (7.10)</td>
</tr>
<tr>
<td>CD Problems</td>
<td>64.45 (11.39)</td>
<td>59.30 (5.50)</td>
</tr>
<tr>
<td><strong>Syndrome Scales</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxious/ Depressed</td>
<td>62.55 (11.11)</td>
<td>61.40 (8.13)</td>
</tr>
<tr>
<td>Withdrawn/Depressed</td>
<td>61.27* (9.45)</td>
<td>55.10* (4.91)</td>
</tr>
<tr>
<td>Somatic Complaints</td>
<td>62.36 (7.27)</td>
<td>57.60 (7.81)</td>
</tr>
<tr>
<td>Social Problems</td>
<td>61.09 (9.91)</td>
<td>60.00 (9.42)</td>
</tr>
<tr>
<td>Thought Problems</td>
<td>65.09 (10.44)</td>
<td>60.60 (7.78)</td>
</tr>
<tr>
<td>Attention Problems</td>
<td>66.18 (10.33)</td>
<td>68.50 (8.82)</td>
</tr>
<tr>
<td>Rule-Breaking Behavior</td>
<td>59.73 (8.98)</td>
<td>57.80 (5.59)</td>
</tr>
<tr>
<td>Aggressive Behavior</td>
<td>69.64* (13.06)</td>
<td>60.60* (5.82)</td>
</tr>
</tbody>
</table>

Note. *p < .15. Standard deviations are in brackets following the means.
Participants were recruited via ads placed in local newspapers; online advertisements posted on FaceBook and Kijiji; posters with pull-tabs displayed on public bulletin boards; brochures circulated to health care facilities, such as hospitals, psychologists’ offices, and doctors’ waiting rooms, and distributed to other health care professionals. Advertisements directed people to the study’s website (http://probioticstudy.com) where more information about the study was available. The website provided the eligibility criteria for participants and a study information sheet explaining details of the study in age-appropriate language for the participants to read (Appendix A).

Any individuals who were interested in the study were directed to complete the online consent form (Appendix B) for the online screening questionnaire (Appendix C). Participants who met the criteria and attended the first meeting with the researcher completed a more detailed consent form before continuing with the study (Appendix D). Several other questionnaires and tests were administered during the study, including: a daily food diary prior to the intervention phase, the Child Behavior Checklist (CBCL), the Screen for Child Anxiety Related Emotional Disorders (SCARED- parent and child versions), the Child Disruptive Behaviour Disorders Rating Scale (DBD - ADHD items only), a Continuous Performance Task, a Word Pair test, a Visual Memory Test, and a weekly questionnaire.

**The Online Screening Questionnaire**

The online screening questionnaire contained items related to the prevalence and severity of the child’s ADHD and/or anxiety symptoms. It also contained questions pertaining to the child’s general health, medical history, and diet. The scores retrieved
from the screening questionnaire established whether the child fell into the anxious group (a total score of 10 or higher on the anxiety questions, computed by totaling the scores of the endorsed items), the ADHD group (an average score of 2.5 or higher on either the hyperactive/impulsive or inattentive symptoms, retrieved by taking the sum of the scores of the endorsed items and dividing it by the number of items, as well as an average score on the other symptom cluster of at least 1.5), or both groups. Children, who met the criteria for the ADHD group, anxiety group, or both groups, were asked to participate in the study.

**The Screen for Child Anxiety Related Emotional Disorders (SCARED)**

The parent and child versions of the SCARED (Birmaher et al., 1999) were used in this study. This measure was used to identify symptoms of anxiety disorders in children. There are 41 items included in this version of the measure: each item asked the individual responding to denote the degree of anxiety symptoms exhibited in a given situation on a scale of zero to two (0=not true or hardly ever true, 1=somewhat true or sometimes true, 2=very true or often true). Each item included statements such as “My child worries about other people liking him/her” for the parent version, or a slightly modified “I worry about other people liking me” for the child version. The parents completed the parent-version of the SCARED while considering their child’s symptoms, and the participant completed the child-version of the SCARED. In cases where the child was too young to read and understand the questions, the researcher would read each item out loud and clarify any confusion with the participant. Parents and participants were asked to only consider the behaviors exhibited during the past week when responding to the statements. The total obtainable scores ranged from zero to 123, where higher scores
denoted higher anxiety levels. The questionnaire provides five subscales, each corresponding to one of five types of anxiety: panic disorder/somatic anxiety, generalized anxiety disorder, separation anxiety disorder, social phobia, and school phobia. For example, a score of eight for items 2, 11, 17, 36 may indicate school avoidance (Birmaher et al., 1999). The SCARED questionnaire has good internal consistency, test-retest reliability, and discriminant validity cross-culturally, and reliably evaluates the existence of anxiety in children (Hale, Crocetti, Raaijmakers, & Meeus, 2010).

**Child Behaviour Checklist (CBCL)**

The CBCL (Achenbach & Rescorla, 2001) is a diagnostic tool that can help to identify a number of childhood mental health conditions, including ADHD and anxiety, as well as unusual or problematic behaviours, among children aged 6 to 18 years. The CBCL contains DSM-oriented scales, which are used to assess the degree to which a child’s characteristics fit the profile of a number of mental health conditions based on the DSM-IV (APA, 2013) diagnostic criteria. The checklist contains 113 items. The parent rates each item based on how much the statement describes their child, using a three-point scale (0 = “not true”, 1 = “somewhat or sometimes true”, 2 = “very true or often true”). The statements include numerous behaviors that the child may demonstrate, such as “acts too young for his/her age”, “clings to adults or too dependent”, and “disobedient at home.” The CBCL was only completed once, during the first visit, as a way of documenting the range of each participant’s symptoms. Inter-rater reliability for the CBCL is high (ranging between 0.93 and 0.96), as is test-retest reliability (0.95), indicating that the CBCL is a valid and reliable tool for assessing the traits associated with childhood mental health issues (Achenbach & Rescorla, 2001).
Child Disruptive Behaviour Disorders Rating Scale (DBD)

The DBD is a 45-item rating scale based on the DSM-IV, describing symptoms of Disruptive Behavior Disorders (conduct disorder, oppositional defiant disorder and ADHD) for children and adolescents. Only the 18-item measure of ADHD symptoms was used for the present study. Each symptom was rated on a 4-point Likert scale (0 = “not at all”, 1 = “just a little”, 2 = “pretty much”, 3 = “very much”). Total obtainable scores range from zero to 54, where higher scores indicate increasing symptom severity. In the DSM-IV, ADHD is grouped into three subtypes: predominantly inattentive, predominantly hyperactive/impulsive, and a combined type, and these types are addressed by separate subscales on the DBD. The DBD has high validity and reliability, and accurately measures symptoms of disruptive behavior in children and adolescents (Friedman-Weineth, Doctoroff, Harvey, & Goldstein, 2009).

The Word Pairs Test

The Word Pairs test assessed verbal learning and memory using a task similar to the Word Pairs subtest of the Children’s Memory Scale (Cohen, 1997). Each of the words comprising the word pairs test used in the present study was matched with the list of words from the Children’s Memory Scale for the number of syllables, as well as the familiarity of the word, the word’s emotional impact, and “concreteness”, according to Toglia and Battig (1978). The test began with a list of word pairs read aloud by the researcher. Subsequently, the researcher gave the child one of the words and the child was asked to identify the associated word. This was repeated for three trials. Finally, the researcher asked the child to recall as many word pairs as they could remember from the list. A point was given for each correct answer. Fourteen word pairs were used for
children aged nine years and above, and ten word pairs for children aged six to eight years. A total score was calculated out of 56 for the older age group, and out of 40 for the younger age group. These scores were then converted to percent correct for data analysis. Four versions of the Word Pairs Test were created for each group and counterbalanced across the four visits for each participant.

**The Visual Memory Test**

This test was created for the current study to assess participants’ ability to remember and recreate simple shapes. This test was a visual analogue of the Word Pairs test. The learning phase involved showing a sequence of cards, each with a pair of shapes on it, to the participant for a few seconds, and then flipping the card around to reveal only one of the shapes (its paired associate was missing). The participant was told to draw the missing shape that completed the pair. Three rounds were completed with the set of cards, in which the child was shown only one of the shapes and asked to draw the missing shape. In the final round, the participants drew as many shape pairs as they could remember. Children aged six to eight were shown six pairs of shapes and children older than eight were shown eight pairs of shapes. One point was given for each correct answer. A total score was calculated out of 32 for children in the older age range, and out of 24 for children in the younger age range. These scores were converted to percent correct prior to data analysis. Four versions of the Visual Memory Test were created and counterbalanced across the four visits for each participant.

**The Weekly Questionnaire**

The weekly questionnaire used in this study, but not part of this thesis, was made by the researchers. The questionnaire was used to record any changes in anxiety, ADHD,
or physical health throughout each week of the study. This questionnaire contained 15 items. Eleven of the items gathered information from the participants about their weekly anxiety (e.g., on a scale of 1-5 the child would answer “How worried or anxious were you overall today?”), gastrointestinal symptoms (e.g., “Did your tummy feel okay today?” or “Was your pooping okay today?”), and ADHD symptoms (e.g., on a scale of 1-5 the child would answer “How easy or hard was it for you to sit still when you were supposed to today?”) (Appendix E). The parents completed four items on the weekly questionnaire, with one question related to their child’s anxiety symptoms, two questions related to ADHD symptoms, and one question asking whether their child attended any psychotherapy or counseling sessions that week (Appendix F).

The Continuous Performance Task

The continuous performance task used in this study, but not part of this thesis, was based on the X/AX task produced by Rosvold, Mirsky, Sarason, Bransome, and Beck (1956). It was used to assess the ability of the participants to maintain sustained and selective attention.

Cortisol Analyses

Analysis of salivary cortisol levels were part of the larger study, but they were not part of this thesis. Saliva samples were obtained at the beginning and end of each phase of the study using a cryovial test tube and straw provided by the researchers. Samples were analyzed for cortisol levels using immunoassay kits purchased from Salimetrics.

Probiotic and Placebo

Lallemand Health Solutions in Montreal supplied the probiotics used in this study. The Probio’Stick contains two probiotic strains of bacteria, Lactobacillus helveticus
R0052 and Bifidobacterium longum R0175. Each packet of probiotics was guaranteed to contain a minimum of 2 billion culture-forming units (CFUs) at the expiry date. The bacteria were microencapsulated to increase their chances of survival when vulnerable to acidic stomach environments and bile salts. Lallemand also supplied the placebo, consisting of the nonmedicinal ingredients contained in the probiotic powder (i.e., fruit flavor, xylitol, maltodextrin, and malic acid), without the probiotics.

**Procedure**

This thesis focuses on the first phase of the study, which took approximately five weeks to complete. Participants completed the eligibility and screening questionnaire, and those who were approved for participation in the study were contacted to schedule their first meeting. The study began with the participants (with the help of their parents) filling out a food diary (Appendix G), as well as a daily questionnaire (Appendices E and F, which is the same as the weekly questionnaire), each day for one week. Each participant and his or her parent/guardian were then scheduled to meet with a researcher for the first time at either Acadia University or the Halifax location. Informed consent regarding participation in the study was obtained and any questions about the study were answered by the researcher. Participants generated a code number, which was then used to identify all materials throughout the study in order to maintain confidentiality.

Parents/guardians then completed the SCARED Parent version and the DBD. On the first visit only, the parent/guardian also filled out an intake questionnaire and the CBCL. The child completed the SCARED Child version, along with the several cognitive measures described above.
Each participant was randomly assigned to receive either the placebo or probiotic within their respective group (i.e., ADHD, anxiety, or both). The study employed a double-blind paradigm. Only the production manager at Lallemand Health Solutions had access to the Lot numbers that would reveal the experimental conditions of the participants. This information was provided only after data analyses had been completed.

Participants were provided with a four-week supply of either the probiotics or placebo and were instructed to consume one packet daily for 28 days. The parent/guardian was also given an $8 Sobeys gift certificate to purchase the necessary products (i.e., milk or ice cream) to take with the powder. In the first visit, two saliva sample tubes were given to collect one baseline saliva sample and another at the end of the first phase. Two instructions sheets were also given with details regarding powder consumption (Appendix H) and saliva sample collection (Appendix I) for each phase of the study. Once a week throughout the entire study, an e-mail was sent to the parents/guardians reminding them to fill out the weekly questionnaire. A reminder e-mail was also sent out two days before each scheduled meeting time at Acadia University and the Halifax location.

After consuming the probiotic or placebo powder for four weeks, participants were asked to return for a second visit, where the same cognitive tests and questionnaires were administered to both the participants and parents. Saliva samples from the first phase were retrieved and stored in a freezer for later analysis and empty packets of the powder from each of the 28 days were retrieved where possible for confirmation of consumption. This completed the first phase. This phase was followed by two additional four-week phases, which are beyond the scope of this thesis and will be described in other reports.
Hypotheses

1. The first hypothesis was that ADHD and anxiety symptom scores (measured by
the DBD, SCARED Parent, and SCARED Child) would improve in the probiotic
group, but not in the placebo group. Specifically, it was hypothesized that:
   a. There would be a significantly greater decrease in scores on the DBD
      (Inattention and Hyperactivity) from visit one to visit two in the probiotic
      condition compared to the placebo condition.
   b. There would be a significantly greater decrease in scores on the SCARED
      Parent, as well as the SCARED Child, from visit one to visit two in the
      probiotic condition compared to the placebo condition.

2. The second hypothesis was that memory would improve in the probiotic group,
   but not in the placebo group. Specifically, it was hypothesized that:
   a. There would be a significantly greater increase in total scores on the
      Verbal Memory (Word Pairs) test from visit one to visit two in the
      probiotic condition compared to the placebo condition.
   b. There would be a significantly greater increase in total scores on the Visual
      Memory (Shape Pairs) test from visit one to visit two in the probiotic
      condition compared to the placebo condition.

3. The third hypothesis was that there would be a significant relation between
   improvements in memory and improvements in ADHD symptoms (DBD Total,
   Hyperactivity, and Inattention scores) and between improvements in memory and
   improvements in anxiety symptoms (SCARED Parent and SCARED Child).
These correlations would be evident in the entire sample, and within each condition (Probiotic and Placebo), as follows:

a. DBD Total scores would be negatively correlated with Verbal Memory and with Visual Memory; that is, a decrease in DBD Total scores would be related to improvement in Verbal and Visual Memory.

b. Inattention scores would be negatively correlated with Verbal Memory and with Visual Memory; that is, a decrease in Inattention would be related to improvement in Verbal and Visual Memory.

c. Hyperactivity scores would be negatively correlated with Verbal Memory and with Visual Memory; that is, a decrease in Hyperactivity would be related to improvement in Verbal and Visual Memory.

d. SCARED Parent scores would be negatively correlated with Verbal Memory and with Visual Memory; that is, a decrease in SCARED Parent scores would be related to improvement in Verbal and Visual Memory.

e. SCARED Child scores would be negatively correlated with Verbal Memory and with Visual Memory; that is, a decrease in SCARED Child scores would be related to improvement in Verbal and Visual Memory.

Results

Description of Data Analyses

Data analyses included seven outcome measures (dependent variables) for each visit (Time 1 and Time 2): the DBD Total score, DBD Hyperactivity and Inattention subscale scores, the SCARED Parent and SCARED Child scores, and the Verbal and Visual memory scores. The data were analyzed using multivariate repeated-measures
analysis of variance (MANOVA) with Condition (Probiotic/Placebo) as the between subjects factor and Visit (Time 1, pre-intervention and Time 2, post-intervention) as the within subjects factor. To test the assumptions of normality, outlier scores were replaced with values two standard deviations away from the mean, and skewness and kurtosis values were examined. In all cases, the skewness and kurtosis values were within the acceptable range (±2), suggesting a normal distribution. Histograms were also examined for normality. In some cases, the histograms did not appear to be normally distributed; in those cases, non-parametric tests (the Mann-Whitney U Test for between group differences, and the Wilcoxon Signed Rank Test for within group differences) were additionally run to confirm the results of the multivariate analyses. For the non-parametric tests, change scores (the difference in score between Time 1 and Time 2) were first calculated for each outcome measure and served as the dependent variables.

In cases where the data were normally distributed and multivariate repeated-measures MANOVAs were run, when the multivariate $F$ was significant, the univariate $F$s were examined. In all cases where the multivariate $F$ was not significant, the univariate $F$s were also all non-significant. However, when the multivariate $F$ was not significant, near significant univariate $F$s are described in case they provide potentially useful information with respect to the larger, on-going study. An alpha level of .05 was used as the cut-off for significance; however, given the small sample size and the exploratory nature of this preliminary report on the first group of participants, $p$ values between .05 and .1 are described as approaching significance, and $p$ values greater than .1 are described as non-significant.
Three separate MANOVAs were carried out: one for the ADHD measures (DBD Total, Hyperactivity, and Inattention), one for the anxiety measures (SCARED Parent and SCARED Child), and one for the memory measures (Verbal Memory and Visual Memory). Three separate MANOVAs were used because each of these groups of outcome variables represents a different construct. Prior to running the MANOVAs, intercorrelations among the variables that were included in each MANOVA were examined to check for collinearity. When the correlation between two variables was greater than .7, only one of the variables was included in the analysis. Following significant multivariate and univariate $F$-tests, paired samples $t$-tests were computed to examine within-subjects effects (i.e., change in scores from Time 1 to Time 2, within each condition).

Correlation analyses were also conducted to examine associations among the outcome measures, overall and separately within each group. Accounting for the small sample size and low statistical power, instead of identifying meaningful correlations by level of statistical significance, moderate correlations ($> .40$), even if not statistically significant, were considered potentially important. Correlations were run separately for Time 1 and Time 2 data in the entire sample, followed by the Probiotic and Placebo conditions, in order to evaluate the associations between the variables and any potentially meaningful differences in correlations within the different conditions and at the two time points.

**CBCL Analyses**

Pearson product-moment correlation coefficients were calculated to compare the baseline measures of anxiety and ADHD with the baseline problem behaviors on the CBCL, as seen in Table 2. Correlations were run to compare the anxiety (SCARED
Table 2
Correlations of CBCL Problem Behavior T-Scores With Anxiety and ADHD Measures

<table>
<thead>
<tr>
<th></th>
<th>SCARED Child</th>
<th>SCARED Parent</th>
<th>DBD Hyperactivity</th>
<th>DBD Inattention</th>
<th>DBD Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCARED Child</td>
<td>1</td>
<td>-.13</td>
<td>-.05</td>
<td>-.11</td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>-</td>
<td>1</td>
<td>-.03</td>
<td>.07</td>
<td>.02</td>
</tr>
<tr>
<td>Externalizing</td>
<td>.08</td>
<td>-.06</td>
<td>.41</td>
<td>.17</td>
<td>.33</td>
</tr>
<tr>
<td>Internalizing</td>
<td>.36</td>
<td>.75**</td>
<td>.15</td>
<td>.30</td>
<td>.26</td>
</tr>
<tr>
<td>Anxiety Problems</td>
<td>.32</td>
<td>.81**</td>
<td>.04</td>
<td>.09</td>
<td>.07</td>
</tr>
<tr>
<td>ADHD Problems</td>
<td>-.25</td>
<td>-.28</td>
<td>.52*</td>
<td>.61**</td>
<td>.68**</td>
</tr>
<tr>
<td>Anxious/Depressed</td>
<td>.37</td>
<td>.82**</td>
<td>.15</td>
<td>.18</td>
<td>.19</td>
</tr>
<tr>
<td>Attention Problems</td>
<td>-.06</td>
<td>-.05</td>
<td>.57**</td>
<td>.80**</td>
<td>.82**</td>
</tr>
</tbody>
</table>

Note: Pearson correlation coefficients (significance 2-tailed) * $p < 0.05$ **$p < 0.01$
Parent and SCARED Child) and ADHD (DBD Total, DBD Inattention, and DBD Hyperactivity) symptom questionnaires with the CBCL scales that corresponded to similar types of symptoms (Externalizing, Internalizing, Anxiety Problems, ADHD Problems, Anxious/Depressed, and Attention Problems). As shown in Table 2, it was found that the SCARED Parent was significantly positively correlated with Internalizing problems, Anxiety Problems, and Anxious/Depressed. As would be expected, this suggests that the SCARED Parent is a valid measure when determining baseline anxiety levels as compared to the CBCL. However, there were no significant correlations between the SCARED Child and any of the CBCL problem behaviors.

As can be seen in Table 2, there were significant positive correlations between DBD Hyperactivity and CBCL ADHD Problems, and DBD Hyperactivity and CBCL Attention Problems. Additionally, there was a moderate positive correlation (although not significant) between DBD Hyperactivity and CBCL Externalizing Behaviors. Significant positive correlations were observed between DBD Inattention and CBCL ADHD Problems, as well as between DBD Inattention and CBCL Attention Problems. Finally, positive correlations were also observed between DBD Total and CBCL ADHD Problems, as well as DBD Total and CBCL Attention Problems. As would be expected, this suggests that the questionnaires used as a baseline measure of ADHD (i.e., DBD Total, Inattention, and Hyperactivity scores) were valid relative to the CBCL when determining baseline ADHD symptom severity. However, none of the DBD measures correlated significantly with the CBCL Externalizing Behaviors composite score.
ADHD Measures

To assess Hypothesis 1a, that ADHD would improve with probiotics but not with placebo, a repeated measures MANOVA was run on the DBD measures of Inattention and Hyperactivity with Condition (Probiotic vs. Placebo) as the between-subjects variable and Time (pre- and post-intervention) as a repeated-measures variable. Prior to running this analysis, it was determined that the DBD Total score was highly correlated with both Hyperactivity and Inattention \((r > .7)\), as would be expected; therefore, the DBD Total score was not included as a dependent measure in this analysis. There was no main effect of Condition \((p > .1)\), indicating that the Probiotic and Placebo groups did not differ on the ADHD measures, collapsed across Time 1 and Time 2, and across Hyperactivity and Inattention. However, there was a main effect of Time \(F(2, 18) = 8.937, p = .002\); collapsed across conditions, DBD Hyperactivity and Inattention scores changed significantly from Time 1 to Time 2. Univariate tests revealed a significant change in Hyperactivity scores, \(F(1, 19) = 15.07, p = .001\), and a near significant change in Inattention scores, \(F(1, 19) = 3.085, p = .095\). As shown in Table 3, paired sample \(t\)-tests indicated that, in partial support of Hypothesis 1a, there was a significant reduction in Hyperactivity scores in both groups, reflecting significant improvements in Hyperactivity in the Probiotic group \(t(9) = 2.763, p = .020\), and in the Placebo group, \(t(9) = 2.774, p = .022\). This improvement in Hyperactivity in the Placebo group is contrary to the hypothesis. Although improvements in Inattention were hypothesized for the Probiotic group, there were no significant changes in Inattention in either the Probiotic group or the Placebo group \((p > .1)\), although inspection of the means reveals that scores post-intervention were slightly lower than baseline in both groups.
Table 3  
**ADHD Symptom Scores (DBD Total, DBD Hyperactivity and Inattention Subtotal): Medians, Means, and Standard Deviations at Time 1 (Baseline) and Time 2 (Post-Intervention), and Difference Scores (Time 2 – Time 1) for Probiotic and Placebo Conditions**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Probiotic</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBD Total Time 1</td>
<td>Median</td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>51.09</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(11.78)</td>
</tr>
<tr>
<td>DBD Total Time 2</td>
<td>Median</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>47.82</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(12.95)</td>
</tr>
<tr>
<td>DBD Total Difference</td>
<td>Median</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.27</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(5.68)</td>
</tr>
<tr>
<td>Inattention Time 1</td>
<td>Median</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>26.55</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(6.38)</td>
</tr>
<tr>
<td>Inattention Time 2</td>
<td>Median</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>25.09</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(6.92)</td>
</tr>
<tr>
<td>Inattention Difference</td>
<td>Median</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>4.20</td>
</tr>
<tr>
<td>Hyperactivity Time 1</td>
<td>Median</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>24.55</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(6.96)</td>
</tr>
<tr>
<td>Hyperactivity Time 2</td>
<td>Median</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>22.73</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(7.25)</td>
</tr>
<tr>
<td>Hyperactivity Difference</td>
<td>Median</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(2.18)</td>
</tr>
</tbody>
</table>

Note: All condition (Probiotic and Placebo) comparisons were non-significant.
Anxiety Measures

A repeated-measures MANOVA was run on Parent and Child SCARED scores to test Hypothesis 1b, that anxiety scores would decrease in the Probiotic condition. The results were not consistent with the hypothesis; there were no significant main effects or interactions (\(p > .1\)) suggesting that there were no changes in anxiety scores. Although the multivariate tests were not significant, one univariate test that approached significance is described here; the univariate \(F\) for the effect of Time, collapsed across Probiotic and Placebo conditions, approached significance for the SCARED Parent measure, \(F(1,19) = 3.467, p = .078\). As shown in Table 4, \(t\)-tests revealed that the mean scores on the Parent SCARED decreased in both groups, but not significantly. Similarly, the Child SCARED decreased only very slightly, and not significantly, from pre- to post-intervention.

Memory Measures

A repeated-measures MANOVA was run on the memory measures with Condition (Probiotic vs. Placebo) as the between-subjects variable and Time (pre- and post-intervention) as a repeated-measures variable in order to test hypotheses 2a and 2b. The dependent variables were Visual Memory and Verbal Memory, where scores were obtained by calculating the percentage of correct answers on the tests. There was no main effect of Condition, \(F(2, 18) = .555, p > .1\), indicating that, collapsed across the time points and type of memory test (visual vs. verbal), there was no difference between the Probiotic and Placebo conditions on the memory measures. There was also no significant effect of Time, \(F(2, 18) = .668, p > .1\), indicating that, collapsed across probiotic and placebo conditions, and type of memory test, the memory scores did not change from Time 1 to Time 2. However, as hypothesized, there was a significant Condition x Time
Table 4
*Anxiety Symptom Scores (SCARED Parent and SCARED Child): Medians, Means, and Standard Deviations at Time 1 (Baseline) and Time 2 (Post-Intervention), and Difference Scores (Time 2 – Time 1) for Probiotic and Placebo Conditions*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Probiotic</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCARED Child Time 1</strong></td>
<td>Median</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>20.55</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(8.74)</td>
</tr>
<tr>
<td><strong>SCARED Child Time 2</strong></td>
<td>Median</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>19.36</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(17.85)</td>
</tr>
<tr>
<td><strong>SCARED Difference</strong></td>
<td>Median</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(13.06)</td>
</tr>
<tr>
<td><strong>SCARED Parent Time 1</strong></td>
<td>Median</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>20.82</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(14.96)</td>
</tr>
<tr>
<td><strong>SCARED Parent Time 2</strong></td>
<td>Median</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>18.64</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(15.04)</td>
</tr>
<tr>
<td><strong>SCARED Parent Difference</strong></td>
<td>Median</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>2.18</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(5.98)</td>
</tr>
</tbody>
</table>

* Difference scores were calculated for each participant, then the means and medians of the difference score distributions were determined.

Note: All Time 1 to Time 2 comparisons were non-significant.
interaction $F(2, 18) = 7.645, p = .004$, indicating that the probiotic and placebo groups differed at Time 1 and Time 2 on the memory measures, collapsed across visual and verbal memory tests. Inspection of the univariate $F$-tests revealed a significant Time x Condition interaction only for Visual Memory $F(1, 19) = 11.135, p = .003$, in support of Hypothesis 2b, and not for Verbal Memory ($p > .1$), rejecting Hypothesis 2a. As shown in Table 5, when paired samples $t$-tests were conducted to determine the extent of the changes in visual memory scores within each condition, partial support for Hypothesis 2b was found. Visual Memory scores improved somewhat from pre- to post-intervention among participants receiving the probiotic with results approaching significance, $t(10) = -2.084, p = .064$, whereas the placebo group’s Visual Memory scores got significantly worse, $t(9) = 2.554, p = .031$. None of the Verbal Memory tests were significant ($p > .1$).

**Correlations**

To test hypotheses 3a, 3b, 3c, 3d, and 3e in relation to the entire sample, Pearson product-moment correlation coefficients were calculated to examine the associations between dependent variables collapsed across conditions (Probiotic and Placebo). Contrary to expectations, there were no significant correlations between any of the variables that were hypothesized, nor did any of the correlations indicate a moderate association ($p > .4$), as shown in Tables 6, 9, 10, and 11.

Pearson product-moment correlation coefficients were then calculated to examine associations between dependent variables when considering Probiotic and Placebo conditions separately. Correlations were computed for the Probiotic condition at Time 1 and Time 2, as shown in Tables 7, 9, 10, and 11, and the Placebo condition at Time 1 and Time 2, as shown in Table 8, 9, 10, and 11.
Table 5
*Memory (Verbal and Visual) Percentage Score Medians, Means, and Standard Deviations at Time 1 (Baseline) and Time 2 (Post-Intervention), and Difference Scores (Time 2 – Time 1) for Probiotic and Placebo Conditions*

<table>
<thead>
<tr>
<th>Measure</th>
<th>Median</th>
<th>Probiotic</th>
<th>Placebo</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Verbal Memory Time 1</strong></td>
<td>Median</td>
<td>50.0</td>
<td>36.6</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>42.79</td>
<td>37.46</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(20.83)</td>
<td>(21.21)</td>
<td></td>
</tr>
<tr>
<td><strong>Verbal Memory Time 2</strong></td>
<td>Median</td>
<td>39.3</td>
<td>38.8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>42.63</td>
<td>39.64</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(22.79)</td>
<td>(22.34)</td>
<td></td>
</tr>
<tr>
<td><strong>Verbal Memory Difference</strong></td>
<td>Median</td>
<td>0.0</td>
<td>-3.6</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>-0.16</td>
<td>2.18</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(21.38)</td>
<td>(26.65)</td>
<td></td>
</tr>
<tr>
<td><strong>Visual Memory Time 1</strong></td>
<td>Median</td>
<td>33.3</td>
<td>50.0</td>
<td>( p = .016 )</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>34.37</td>
<td>49.69</td>
<td>( p = .013 )</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(9.83)</td>
<td>(15.49)</td>
<td></td>
</tr>
<tr>
<td><strong>Visual Memory Time 2</strong></td>
<td>Median</td>
<td>40.6</td>
<td>35.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>43.66</td>
<td>33.44</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(17.72)</td>
<td>(22.34)</td>
<td></td>
</tr>
<tr>
<td><strong>Visual Memory Difference</strong></td>
<td>Median</td>
<td>12.5</td>
<td>-15.6</td>
<td>( p = .013 )</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>9.29</td>
<td>-16.25</td>
<td>( p = .003 )</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>(14.78)</td>
<td>(20.12)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The median was that of all the individual difference scores, rather than the Time 1 median minus Time 2 median.

*Difference score were calculated for each participant, then the means and medians of the difference score distributions were determined. Positive difference scores indicate improvement. NS = Not Significant
Table 6  
*Correlations Between Dependent Variables at Time 1 and Time 2 in the Entire Sample*

<table>
<thead>
<tr>
<th></th>
<th>DBD TOTAL</th>
<th>Hyperactivity</th>
<th>Inattention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>.02</td>
<td>-.03</td>
<td>.07</td>
</tr>
<tr>
<td>SCARED Child</td>
<td>-.11</td>
<td>-.13</td>
<td>-.05</td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>-.06</td>
<td>-.22</td>
<td>.11</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>-.11</td>
<td>-.32</td>
<td>.10</td>
</tr>
<tr>
<td><strong>Time 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>.03</td>
<td>.01</td>
<td>-.07</td>
</tr>
<tr>
<td>SCARED Child</td>
<td>-.05</td>
<td>-.23</td>
<td>.11</td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>.09</td>
<td>-.04</td>
<td>.19</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>.07</td>
<td>-.12</td>
<td>.29</td>
</tr>
</tbody>
</table>

Note: Pearson correlation coefficients (significance 2-tailed). None of the correlation coefficients was meaningful (i.e., all < r = .4).
Table 7
*Correlations Between Dependent Variables at Time 1 and Time 2 in the Probiotic Condition*

<table>
<thead>
<tr>
<th></th>
<th>DBD TOTAL</th>
<th>Hyperactivity</th>
<th>Inattention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>.16</td>
<td>.05</td>
<td>.24</td>
</tr>
<tr>
<td>SCARED Child</td>
<td>-.15</td>
<td>.01</td>
<td>-.29</td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>.15</td>
<td>-.03</td>
<td>.31</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>-.01</td>
<td>-.11</td>
<td>.11</td>
</tr>
<tr>
<td><strong>Time 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>.16</td>
<td>.14</td>
<td>.15</td>
</tr>
<tr>
<td>SCARED Child</td>
<td>-.17</td>
<td>-.28</td>
<td>-.03</td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>.09</td>
<td>.12</td>
<td>.04</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>.15</td>
<td>-.03</td>
<td>.31</td>
</tr>
</tbody>
</table>

Note: None of the correlation coefficients was meaningful (i.e., all \(< r = .4\)).
Table 8
*Correlations Between Dependent Variables at Time 1 and Time 2 in the Placebo Condition*

<table>
<thead>
<tr>
<th></th>
<th>DBD TOTAL</th>
<th>Hyperactivity</th>
<th>Inattention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>-.30</td>
<td>-.27</td>
<td>-.22</td>
</tr>
<tr>
<td>SCARED Child</td>
<td>-.11</td>
<td>-.30</td>
<td>.06</td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>-.34</td>
<td>-.54*</td>
<td>-.09</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>-.15</td>
<td>-.62*</td>
<td>.22</td>
</tr>
<tr>
<td><strong>Time 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>-.45*</td>
<td>-.31</td>
<td>-.45*</td>
</tr>
<tr>
<td>SCARED Child</td>
<td>.03</td>
<td>-.24</td>
<td>.26</td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>.07</td>
<td>-.25</td>
<td>.32</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>-.11</td>
<td>-.42*</td>
<td>.20</td>
</tr>
</tbody>
</table>

Note: Pearson correlation coefficients (significance 2-tailed). * $r > (+/-) .4$ is considered to be a moderate correlation. No correlations were statistically significant.
Table 9
Correlations Between SCARED Parent and SCARED Child Scores, and Verbal and Visual Memory Percent Scores at Time 1 and Time 2 in the Entire Sample, the Probiotic Condition, and the Placebo Condition

<table>
<thead>
<tr>
<th></th>
<th>Time 1</th>
<th></th>
<th>Time 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCARED Parent</td>
<td>SCARED Child</td>
<td>SCARED Parent</td>
<td>SCARED Child</td>
</tr>
<tr>
<td>Entire Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>-.35</td>
<td>-.28</td>
<td>-.11</td>
<td>-.13</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>.09</td>
<td>-.05</td>
<td>.12</td>
<td>.00</td>
</tr>
<tr>
<td>Probiotic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>-.62*</td>
<td>-.83*</td>
<td>-.11</td>
<td>-.44</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>.10</td>
<td>-.30</td>
<td>.05</td>
<td>-.27</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal Memory</td>
<td>.04</td>
<td>.06</td>
<td>-.13</td>
<td>.46</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>.34</td>
<td>.19</td>
<td>.14</td>
<td>.30</td>
</tr>
</tbody>
</table>

Note: Pearson correlation coefficients (significance 2-tailed). * p < .05
Table 10  
*Correlations Between Inattention and Hyperactivity Scores at Time 1 and Time 2 in the Entire Sample, the Probiotic Condition, and the Placebo Condition*

<table>
<thead>
<tr>
<th></th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inattention</td>
<td>Inattention</td>
</tr>
<tr>
<td><strong>Entire Sample</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>.42</td>
<td>.57**</td>
</tr>
<tr>
<td><strong>Probiotic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>.56</td>
<td>.67*</td>
</tr>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>.32</td>
<td>.47</td>
</tr>
</tbody>
</table>

Note: Pearson correlation coefficients (significance 2-tailed). *p < .05 **p < .01
Table 11
Correlations Between SCARED Parent and SCARED Child Scores at Time 1 and Time 2 in the Entire Sample, the Probiotic Condition, and the Placebo Condition

<table>
<thead>
<tr>
<th></th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCARED Child</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entire Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>.59**</td>
<td>.52*</td>
</tr>
<tr>
<td>Probiotic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>.65*</td>
<td>.69*</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCARED Parent</td>
<td>.64*</td>
<td>.23</td>
</tr>
</tbody>
</table>

Note: Pearson correlation coefficients (significance 2-tailed). * p < .05 ** p < .01
Contrary to Hypothesis 3a; that DBD Total scores would be negatively correlated with Verbal Memory and with Visual Memory, there were no significant or moderate ($r > .4$) correlations between variables in the Probiotic condition at baseline or post-intervention, as shown in Table 7. However, as shown in Table 8, a moderate negative correlation ($r > -.4$) was found between DBD Total and SCARED Parent scores in the Placebo condition at Time 2, suggesting that, as ADHD symptoms improved, anxiety symptoms, as rated by the parent, worsened (and vice versa) following placebo administration.

Hypothesis 3b was also rejected. Inattention scores were not negatively correlated with Verbal Memory or with Visual Memory in either condition or at either time point, as shown in Tables 7 and 8. This suggests that child Inattention, as rated by the parent, did not appear to affect performance on verbal or visual memory tasks. Contrary to expectations, the correlations between Verbal Memory/Visual Memory and Inattention, aside from the Placebo condition at Time 1, were all in a positive direction (although none were significant). Interestingly, a moderate (but non-significant) negative correlation was found between Inattention and SCARED Parent scores in the Placebo condition at Time 2, as shown in Table 8. This suggests a trend toward an inverse relationship; that is, as Inattention scores change, SCARED Parent scores change in the opposite direction.

Contrary to hypothesis 3c, Hyperactivity scores were not negatively correlated with Verbal Memory or with Visual Memory; that is, child hyperactivity, as rated by the parent, did not seem to impact performance on verbal or visual memory tasks. Nevertheless, within the Placebo condition, there were moderate negative correlations found between Hyperactivity and Verbal Memory at Time 1, and Hyperactivity and
Visual Memory at Time 1, as well as Hyperactivity and Visual Memory at Time 2, as shown in Table 8. These correlations indicate, contrary to the hypotheses, that there was a trend toward an association between increased Hyperactivity and poorer verbal and visual memory performance (or vice versa), but only in the Placebo condition. Although the remaining correlations were not significant, all correlations between Hyperactivity and both Verbal and Visual Memory, aside from the Probiotic condition at Time 2, were in the negative direction, as predicted in Hypothesis 3c.

Additionally, there was a significant positive correlation between Hyperactivity and Inattention at Time 2, and a near significant positive correlation at Time 1, in the Probiotic condition (as seen in Table 10). There was also a moderate (but non-significant) positive correlation between Hyperactivity and Inattention in the Placebo condition, but only at Time 2 (as seen in Table 10). When considering the entire sample, there was a moderate positive correlation between Inattention and Hyperactivity at Time 1, and a significant positive correlation at Time 2, shown in Table 10. As could be expected, this suggests that as Hyperactivity scores change, Inattention scores change in the same direction, but mainly within the Probiotic condition.

Hypotheses 3d and 3e, stating that both SCARED Child and SCARED Parent scores would be negatively correlated with Verbal Memory and with Visual Memory, were partially supported. As shown in Table 9, there was a significant negative correlation between SCARED Parent scores and Verbal Memory, and SCARED Child scores and Verbal Memory within the Probiotic condition, but only at Time 1 (baseline). This suggests that increased anxiety at baseline, as rated by the parent and child, was associated with poorer verbal memory (and vice versa), but only in the Probiotic condition.
condition. However, as seen in Table 9, there was a moderate (but non-significant) negative correlation between SCARED Child and Verbal Memory scores at Time 2 within the Probiotic condition. This suggests that as anxiety symptoms improved, Verbal Memory scores improved (and vice versa) after taking the probiotic. Surprisingly, there was a moderate (but non-significant) positive correlation between SCARED Child scores and Verbal Memory at Time 2 in the Placebo condition, as seen in Table 9. This would suggest that as anxiety symptoms got worse, as reported by the child, Verbal Memory scores improved (and vice versa) after taking the placebo. As seen in Table 9, correlations between Visual Memory and both SCARED Parent and SCARED Child scores at Time 1 and Time 2, as well as Verbal Memory at Time 1, in the Placebo condition were also all positive, contrary to expectations.

Furthermore (as would be expected), in the Probiotic condition, there were significant positive correlations between SCARED Parent scores and SCARED Child scores at Time 1 and Time 2, as shown in Table 11. This was also the case for the Placebo condition, but only at Time 1. When considering the entire sample (collapsed across Probiotic and Placebo conditions), positive correlations between SCARED Parent and SCARED Child were seen at Time 1 and Time 2. This indicates that, the parent’s rating of their child’s anxiety improved, the child’s rating of their anxiety also improved (and vice versa).

Discussion

In the present day, as mental health issues are becoming more recognized, people may try to avoid the potentially negative side effects of medications by exploring alternative treatment options. Alternative or complementary health products are widely
used, with over five million Canadians using them in the past year (Statistics Canada, 2005), spending approximately $2.35 billion per year (Born & Laupacis, 2011). Probiotics represent one such alternative/complementary natural health product.

The aim of the current study was to examine the effects of probiotics versus placebo on memory and symptoms of ADHD and anxiety in children. Extensive research has shown a positive relationship between probiotics and mental health (Desbonnet et al., 2008; Bravo et al., 2011; Messaoudi et al., 2011; Pärtty et al., 2015). Only a small sample size was available for the current study (eleven children in the probiotic group, and ten children in the placebo group), which made it difficult to achieve the high statistical power that was desired. However, even with this small sample size, significant results were obtained and trends toward significance were observed and reported.

Effects of Probiotics on Anxiety and ADHD Symptoms

As hypothesized, there was a significant improvement in parents’ ratings of hyperactivity among those who had consumed the probiotic compared to the placebo between the first visit (pre-intervention) and the second visit (post-intervention). However, contrary to expectations, there were no significant improvements in ratings of inattention in either the probiotic group or the placebo group. Overall, when looking at the parent-rated total ADHD symptoms scores, there were no significant differences between the probiotic and placebo conditions, presumably due to the lack of probiotic effect on attention. A significant improvement in hyperactivity after taking probiotics for four weeks may suggest a more specific relationship between probiotics and the hyperactive component of ADHD, as opposed to the attention component. This is inconsistent with the results of Harding and colleagues (2003), although there were many differences
between their study and ours. They found significant attention improvements in children with ADHD who were prescribed dietary supplements including probiotics. However, the specific effects of probiotics on attention in their study were difficult to isolate since the supplements included a number of additional ingredients (e.g., multiple vitamin, multiple mineral, phytonutrients, essential fatty acids, amino acids, and phospholipids). Our results provide evidence in support of probiotics having a more significant effect on hyperactivity in particular, but further evidence is needed to isolate the effects of probiotics on the hyperactivity and inattention components of ADHD separately.

An improvement in hyperactivity in both the probiotic and placebo conditions indicates the presence of a placebo effect. This occurrence is well recognized in research with studies involving treatment procedures (Rutherford et al., 2012). Since pharmaceutical treatments for anxiety and ADHD are not recommended for children under the age of 18 (Health Canada, 2004) and can produce undesirable effects (Ferguson, 2001), there is high potential for parents to be very hopeful that their child would show improvements in his or her symptoms when using therapies that have no negative physiological side-effects. Furthermore, the possible placebo effects seen in this study could have emerged from expectations of favorable health benefits associated with probiotics that the participants/parents may have seen in product advertisements (e.g., yoghurt). For participants, this may have led to the belief that their symptoms would improve. This could have also led parents to have a predetermined idea that the probiotics would be beneficial, leading them to detect improvement even though their child was taking the placebo.
It was hypothesized that there would be a significantly greater improvement in anxiety, as measured by the SCARED Parent and SCARED Child questionnaires, from pre- to post-intervention in the probiotic group compared to the placebo group. Contrary to this hypothesis, no significant differences were found between the probiotic condition and the placebo condition. However, anxiety symptoms improved slightly (i.e., the trend was approaching significance, but was not statistically significant) from pre- to post-intervention in both probiotic and placebo conditions. This slight improvement in anxiety indicates that the results are proceeding in the right direction; that is, perhaps with a larger sample size, the decrease in anxiety seen in the probiotic condition would become statistically significant. The lack of a significant probiotic effect on anxiety contrasts with the results of Messaoudi et al. (2011). There are a number of reasons for the differences in outcomes. Importantly, the participants in their study were adults whereas our participants were children. Since the participants in the study by Messaoudi et al. were all adults, they could have possibly given more accurate depictions of their stress and anxiety symptoms. In the present study, not only did parents fill out a questionnaire based on how they perceived their child’s symptoms through subjective observations, younger children in this study required the help of parents to complete their questionnaires. Therefore, self-report measures for children may not reflect the symptoms as accurately, and may be less reliable than self-reports from adults. Additionally, different anxiety outcome measures were used in the two studies.

Effects of Probiotics on Memory

At first glance, the hypothesis that probiotics would have a beneficial effect on visual memory compared to placebo appeared to be supported. However, upon further
analysis, it was determined that, although there was somewhat of an improvement in visual memory among participants receiving the probiotic, the apparent probiotic effect was largely due to a significant worsening of visual memory among participants receiving the placebo. That is, although visual memory improved among participants receiving the probiotic, the effect only approached significance; on the other hand, the deterioration in memory performance among participants receiving the placebo was significant. Contrary to the hypothesis, no change in performance on the verbal memory tasks was seen among either the probiotic or placebo groups.

Animal research supports the link between probiotic consumption and improved visual memory. Studies where rats were subjected to maze tasks after consuming probiotic supplements showed beneficial effects on memory (Bercik et al., 2011; Davari, Talaei, Alaei, & Salami, 2013; Matthews & Jenks, 2013). Rats who completed these mazes used their visual memory, more specifically visuospatial memory, to recall how to maneuver through a radial arm maze or find an underwater platform in a swimming task. The findings of the present study are somewhat consistent with the animal research with respect to visual memory; these studies have little to say about the effects of probiotics on verbal memory.

**Correlations Among the Dependent Measures**

No associations were found between the hypothesized variables when considering the entire sample (collapsed across probiotic and placebo conditions). However, correlations within each time point and between conditions (probiotic and placebo) were also considered. It was hypothesized that hyperactivity and inattention would negatively impact performance on the memory tasks. However, when the relation between overall
symptoms of ADHD and memory performance was examined for the visual and verbal memory tasks, correlational analyses revealed no significant effects; that is, parent-rated decreases in overall ADHD symptoms were not associated with improvements on the verbal or visual memory tasks.

The hypothesis that parent-rated improvements in inattention would be associated with better performance on the verbal and visual memory tasks was also not supported; inattention scores were not associated with memory performance. In fact, contrary to the hypotheses, the majority of the correlations between verbal/visual memory performance and inattention were positive, albeit insignificant, indicating that, if anything, memory performance improved slightly as parent-rated inattention worsened.

Additionally, contrary to the hypotheses, parent-rated hyperactivity did not appear to impact performance on the verbal or visual memory tasks. It was expected that each of the symptom components of ADHD (inattention, hyperactivity, and combined) would improve after taking the probiotics, and parallel improvements in memory (verbal and visual) would also be seen, because the child would be more attentive and calm when learning and recalling the word and shape pairs. It is likely that in the one-to-one setting of the lab used to test participants, hyperactivity and inattention were kept in check; that is, the child was fully engaged. In a different setting, hyperactivity and inattention may be more obvious and have a bigger impact on the tasks that the child is doing. Additionally, as previously mentioned, parents may not have the most objective responses when reporting their child’s symptoms. This is particularly true when considering the fact that parents are unable to reflect on the child’s symptoms and behaviors at school, where inattention and hyperactivity are often most prevalent.
Furthermore, perhaps hyperactivity in particular did not impact verbal or visual memory because, even though a child may be hyperactive, it does not mean they are not listening and absorbing information. Research by Sonuga-Barke, Taylor, and Heptinstall (1992) suggests that memory deficits in children with hyperactivity problems are because of a lack of effortful processing. It was found that when hyperactive and non-hyperactive children were told to spend the same amount of time examining visual stimuli (i.e., the children had 30 seconds to look at the drawings), as opposed to being given as much time as they desired, the hyperactive children performed just as well as the non-hyperactive children. Perhaps significant increases in memory were not related to decreases in hyperactivity in the present study because of the way the memory tests were administered. This suggests that task demands can dampen the effects of hyperactivity on memory. Although not specifically stated by Sonuga-Barke et al., it could be possible that this strategy in which each child receives a fixed amount of time to look at each stimulus may also provide a more clear negative relationship between inattention and memory.

In the placebo condition at pre- and post-intervention periods, there was a moderate association between visual memory and hyperactivity, where visual memory improved as hyperactivity decreased (and vice versa), but this trend was not significant due to the small sample size. This trend was also seen at the pre-intervention period for verbal memory. This may represent chance findings, because it is not clear why hyperactivity would impact verbal memory only in the placebo condition and not in the probiotic condition. However, since this study examined a large number of correlations, there was an increased chance of making a Type II error. This was especially true when examining correlations that were not statistically significant, but were indicating a
moderate relationship between two variables. Therefore, this suggests that one cannot put any emphasis on such unexpected contrary correlations.

A significant positive association between hyperactivity and inattention was discovered in the probiotic condition post-intervention, and there was a near significant association at baseline. These findings were also true when considering the entire sample (collapsed across probiotic and placebo conditions). This would be a logical finding when examining children with ADHD, because it indicates that as their inattention scores increase or decrease, their hyperactivity scores change in the same direction. This association between hyperactivity and inattention may also suggest that most children in this sample had the combined type of ADHD, rather than predominantly inattentive or predominantly hyperactive, since both scores changed together. This finding is representative of the population because the combined type (where no type of symptom is predominant over the other) is the most common type of ADHD among children (Froehlich et al., 2007).

The hypothesis that child anxiety, according to both the parent and participant reports of the child’s anxiety symptoms (i.e., SCARED Child and SCARED Parent scores), would negatively impact verbal and visual memory was partially supported. Verbal memory was significantly associated with anxiety symptoms in the probiotic condition, but only at baseline. This suggests that as reported anxiety symptoms increased at baseline, verbal memory performance decreased (and vice versa), but only among children in the probiotic condition. This is consistent with research stating that memory is often poorer in children with anxiety compared to non-anxious controls (Darke, 1988). Moreover, if children had high anxiety levels, meeting with the researchers could have
caused their anxiety to rise. With high anxiety at the time of testing, it would make sense that these children would be less able to think about the task at hand because of worry or fearful thoughts. This in turn may cause memory scores to be low. At post-intervention, the relation between anxiety and memory may not have been significant because the children knew what to expect and, thus, had lower levels of anxiety, dampening the previous association between memory and anxiety levels. Perhaps the relationship among these variables was stronger for the verbal memory than the visual memory because the verbal memory task required the children to actually speak out loud, rather than just silently drawing shapes on a piece of paper. Speaking out loud to answer memory questions may have been more anxiety-provoking for the children, thereby resulting in a stronger relationship between anxiety and memory performance. Why this association occurred only in the probiotic condition at baseline is unclear. However, it may be related to the size of the participant pool. Perhaps, when visits 3 and 4 are included in future analyses, this effect will be clarified.

In the probiotic condition at the post-intervention period, there was a moderate association (but not statistically significant, due to the small sample size) between anxiety symptoms (as reported by the participant) and performance on the verbal memory task. This relationship is in the direction that was hypothesized; i.e., as anxiety symptoms reported by the participant increased or decreased, their verbal memory changed in the opposite direction. Similar relations between anxiety (parent and child reports) and visual memory were observed among participants in the placebo condition at both the pre- and post-intervention visit, and between anxiety and verbal memory at the post-intervention visit. This is a surprising finding because, even though it is in the placebo group, we
would not expect increases in anxiety to be associated with better performance on the verbal memory task (and vice versa). However, it is possible that, as mentioned previously, among participants with higher anxiety levels during the week prior to the visit, knowing what to expect during the visit may have reduced their anxiety to a level that was beneficial for this type of task.

Overall, there was a strong relation between parent and child ratings of the child’s anxiety levels. That is, the severity of anxiety symptoms reported by the child was similar to the severity of anxiety symptoms reported by the parent of the child. In other words, as parents’ reports of their child’s anxiety changed, the child’s ratings of their anxiety symptoms changed to a similar degree. This indicates that parent and child have similar perceptions of the child’s anxiety symptoms. This is consistent with Hale, Crocetti, Raaijmakers, and Meeus (2010) who claimed that the SCARED questionnaire has good internal consistency, test-retest reliability, and discriminant validity. However, while this was true for the probiotic group at both pre- and post-intervention visits, it was true for the placebo group at the pre-intervention visit only; for some reason, this relation between parent and child ratings was not present in the placebo group at the post-intervention visit. The differing parent and child anxiety ratings in the placebo group post-intervention may reflect some as yet unknown process that may come to light once the study is complete, or it may be a case of Type II error due to the very small sample size (only ten participants in this group).

**CBCL Findings**

In order to identify any differences in mental health problems and behaviors between the participants in the probiotic and placebo groups at baseline (i.e., any
differences between groups that were not eliminated by random assignment), the groups were compared on the various subscales of the CBCL. As hypothesized, there were no significant differences in problem behaviors between the probiotic and placebo conditions at baseline. This indicates that random assignment was successful in that participants in both conditions showed similar levels of psychopathology on the CBCL subscales. However, there were differences between the probiotic and placebo groups’ scores that were approaching significance on several subscales (Externalizing Problems, Somatic Problems, ODD Problems, Withdrawn/ Depressed, and Aggressive Behavior). The mean T-scores for all of the near-significant problem behaviors were higher in the probiotic condition (with four variables that were in the clinical range, and two variables that were in the borderline clinical range), compared to the placebo condition (with three variables in the normal range, and three variables in the borderline clinical range). Specifically, participants in the probiotic condition had somewhat more severe Externalizing Problems, Somatic Problems, ODD Problems, Aggressive Behavior, and they were more withdrawn/ depressed than participants in the placebo condition at baseline. Benton et al. (2007) found that the severity of the initial condition of the subject had an effect on the ability to observe significant changes associated with probiotic administration. In their study, significant improvements in mood were seen after taking the probiotics only in the bottom third of participants whose mood was initially poor. It is possible that similar effects may be observed in our study once it is complete, but at the present time, the sample size is too small to carry out the relevant analyses.

The CBCL (Achenbach & Rescorla, 2001) was administered to examine the levels of a variety of emotional/behavioural symptoms in the participants, in addition to ADHD
and anxiety. Discussion of other mental health symptoms is beyond the scope of this thesis, but correlations were run to compare the anxiety (SCARED Parent and SCARED Child) and ADHD (DBD Total, Inattention, and Hyperactivity) symptom questionnaires with the CBCL subscales that measured similar symptoms (the Externalizing, Internalizing, Anxiety Problems, ADHD Problems, Anxious/Depressed, and Attention Problems subscales). These correlations confirmed that the baseline measures of anxiety and ADHD were valid with respect to the CBCL. It was found that, excluding the SCARED Child, all of the measures of anxiety and ADHD were significantly associated with the relevant CBCL subscales.

None of the DBD subscales (Hyperactivity, Inattention, and ADHD total score) significantly correlated with the CBCL Externalizing Behaviors scale. However, this is not completely unexpected because the Externalizing Behaviors scale is a composite score that is comprised of other types of behaviour problems, namely oppositional-defiant disorder (ODD) and conduct disorder (CD). The lack of relation between the DBD ADHD scales and the CBCL Externalizing Problems scale suggests that, among the children in our sample, ODD and CD symptoms were uncommon, or, if present, they were not associated with ADHD (less likely).

It was also found that the SCARED Child did not significantly correlate with any of the CBCL subscales related to anxiety. This was surprising, since the parent and child SCARED scores were highly correlated at baseline in both the probiotic and placebo conditions, and the parent version of the SCARED was significantly correlated with the CBCL anxiety subscales (Internalizing, Anxiety Problems, and Anxious/Depressed). The most likely explanation for the strong correlations among the CBCL anxiety scales and
the parent SCARED but not the child SCARED is that the parents of the participants completed both the SCARED Parent and the CBCL. Therefore, we would expect them to give similar ratings on the two measures. There is no child version of the CBCL, but had there been, we would expect similar correlations between the child’s own ratings of their anxiety. Nevertheless, these results contrast with the results of Muris, Dreessen, Bögels, Weckx, and van Melick (2004) who found that the SCARED-R scores correlated significantly with the CBCL Internalizing Problems. However, Muris et al. used the revised 66-item SCARED. Therefore, perhaps the SCARED Child measure used in this study differed sufficiently from the version used by Muris et al. to result in a lack of correlation with the anxiety scales of the CBCL.

Limitations

The symptom questionnaires used in this study required the participants to subjectively reflect upon their own symptoms, as well as parents to subjectively report on their child’s symptoms. Such personal accounts and opinions of symptom changes rely on memory for the child’s emotions and behaviour, and, under the study conditions, parent and child responses could be inaccurate, biased, and influenced by the most salient and memorable but perhaps rare moments. In addition to questionnaires, physiological measures may produce more objective and accurate information, and they could be used to help confirm the claims made in the self-report measures. Although saliva samples were collected from the participants at the beginning and end of each phase in the study in order to examine cortisol levels, they could not be analyzed until all samples had been received, because all four samples from each participant had to be run at the same time, and approximately 60 samples had to be analyzed at once. Therefore, for this study in
particular, it may have been beneficial to use a different physiological measure that could be analyzed more easily.

The time of day that participants were tested varied throughout the study. Strong attempts were made to have each visit with each participant scheduled at approximately the same time each day, but unfortunately this was not possible for every participant. If the participant was tested in the late afternoon, their attention span and restlessness may have been higher after being at school all day, as opposed to a morning and/or weekend visit. Additionally, the time of year that participants were tested could have also affected their anxiety and/or ADHD symptoms. Since participants were recruited throughout the summer until January, those participants who were tested in the summer or over Christmas break may have been less anxious because they were not in school. Those who were tested during the regular school year could have had elevated anxiety levels (if school is a trigger of their anxiety), or more inattentiveness after exhausting their attention span during school hours.

Dietary restrictions were clearly outlined at the beginning of the study (e.g., to abstain from probiotic yogurt, kefir, most uncooked cheese, etc.). Some participants may not have followed these guidelines as strictly as required. If the participants in the placebo condition, for example, consumed off-limits foods containing probiotics, these participants could have added beneficial bacteria to their diet. This would have made it hard to distinguish between those in the probiotic condition and those who were supposed to be in the placebo condition, but are getting the probiotics from their diet. Alternatively, it could cause problems if participants in the probiotic group were straying from dietary restrictions and consuming probiotic rich foods as well. If these participants were getting
more probiotics than others in the study, a higher amount of probiotics could possibly have led to better outcomes that the others did not experience with their lower dose of probiotic.

Another possible reason for insufficient findings could have been the participants’ uncertainty surrounding the time frame they were supposed to use when rating their past symptoms. Although the children were told to only consider their symptoms during the past week when completing the questionnaires, it may have been difficult to keep that in mind when answering the questions. If children recognize certain questions as being a common occurrence for them throughout their life, they will most likely rate it high without specifically considering the question as it applies to the past week. If children were taking probiotics, they may have been answering the questions based on how they typically feel, and may not have considered or reflected upon the more recent positive effects of the probiotics, if there had been any. This could have made it more difficult to find reductions in anxiety on the SCARED questionnaires, and could be an explanation as to why no meaningful results were found.

**Future Research**

It is likely that the results did not support the hypotheses in some cases due to the small sample size. This study had a sample size of only 21 participants (11 in the probiotic condition and 10 in the placebo condition); therefore, there was limited statistical power for finding significant differences. This makes the significant results that much more compelling. In those instances where the results approached significance, they may reach significance with the increased sample size (100 participants) once the study is complete.
Subsequent to retrieving a larger sample size, it would be beneficial to analyze groups of participants that have ADHD, anxiety, or both, separately. This way, it would be much easier to isolate the effects of the probiotics on the specific symptoms related to each disorder, rather than to include those who only have ADHD, for instance, in with the anxiety analyses. Perhaps this would show that the effects of probiotics are more specific to one disorder over the other.

When the parents were answering the questionnaires, they were only able to account for symptoms changes in their children with respect to behavior at home. The American Academy of Pediatrics (2000) has shown that teacher ADHD questionnaires in particular have displayed strong responsiveness and individuality, to the extent that they can accurately distinguish between children with ADHD and children without ADHD. It also suggests that the assessment of ADHD actually necessitates evidence directly acquired from teachers. Ratings from teachers are also extensively used in research to measure the results related to treatment (Evans, Axelrod, & Langberg, 2004). With that said, it may be beneficial to collect reports from teachers in future research as well. Since children spend a substantial amount of each week in the classroom, and symptoms are often most prominent when children are asked to stay seated and attentive for long periods of time, teachers would be able to provide important insight into symptom changes. Another benefit to receiving input from teachers is that teachers are able to compare the child’s behaviors with children their age who do not have the disorder. This may allow for a better evaluation of their symptoms.

Participants in this study were taking a wide range of medications to treat their anxiety and ADHD symptoms. Although it was required that participants not change
medication type or dosage during the study period, the fact that some individuals were taking medications and some were not, may have caused some discrepancies. For instance, those on medications may already exhibit decreased symptom severity, or may experience other side-effects that are not due to their disorder, but rather due to the medication (i.e., gastrointestinal disturbances) (Health Canada, 2004; Ahmann et al., 1993). In future research, it may be useful to consider only participants who are not taking medications used to treat ADHD or anxiety for participation in the study. Despite the fact that this may make it more difficult to recruit as many children for the study, it would allow for a more direct observation of the precise link between probiotics and symptoms of ADHD/ anxiety.

Reflecting back to the overall purpose of the study, the main hypothesis that probiotics would improve memory and symptoms of ADHD and anxiety in children compared to placebo was partially supported, with decreased hyperactivity and improved visual memory occurring in the probiotic condition. Other hypothesized probiotic effects that were not significant were observed to be moving in the right direction. Further research is needed in this area for meaningful findings to emerge. Certain limitations in this study (e.g., small sample sizes, subjective self-report measures, dietary deviations, etc.) may have contributed to the lack of significant findings. In future research it would be beneficial to retrieve questionnaires from teachers, recruit a large sample, and more closely account for medication use, in order to increase the likelihood of uncovering more accurate results.


Appendix A

Information and Consent Form for Child Participants

THE EFFECTS OF A PROBIOTIC SUPPLEMENT ON
SYMPTOMS OF ADHD AND ANXIETY

Department of Psychology, Acadia University

When children come in for their first appointment they will be asked to read and sign this form. Children unable to read will have the information explained to them in language they understand.

Researchers

Dr. Susan Potter, Supervisor, email: susan.potter@acadiau.ca, tel: (902) 585-1220

Dr. Mark Johnston, Physician researcher, email: markerj1@me.com, tel: (902) 679-0536

Dear participating child,

You and your parents have agreed to be a part of a research project called “The effects of a probiotic supplement on the symptoms of ADHD and anxiety in children”. You have signed up for this research because sometimes you feel anxious (scared or nervous), or you want to be moving and have trouble sitting still, waiting your turn, or paying attention.

You will be in the study for thirteen weeks. For the first week, you and your parents will keep track of what you eat and how your stomach is feeling. Then you will take a special sweet-tasting powder, mixed in a glass of milk or bowl of ice cream, every day for four weeks, take a four-week break, then take the special powder again for four weeks. For some of those weeks, the special powder will have something call probiotics in it. Probiotics are found in nature and they might help you feel less nervous or be able to sit still and pay attention better.

You will meet with a researcher four times – at the beginning, twice in the middle, and at the end of the study. Each week we will ask you to answer some questions about how your tummy feels, and about your poop, and about how much you were worried that day, if you had trouble staying still and paying attention, and things like that. You can ask the researcher questions at any time when you meet them, or you can send us an email (or ask your parents to send an email) if you have questions at other times. If you do not want to be a part of this research anymore, tell your parents or the researcher how you feel and you can stop.

Every month there will be a prize draw. You will be entered and you could win a prize (like a toy or a movie pass). If you finish all thirteen weeks of the research, you will get 50 dollars at the end.

Thank you for participating!

Sincerely,

Dr. Susan Potter                Dr. Mark Johnston
Research Coordinator            Physician researcher

Please sign indicating you have read this form:

Name of Child: _________________________________
Signature of Child: _________________________________ Date: ___________________

Signature of Researcher: _____________________________ Date: ___________________
Appendix B
Online Consent Form for Eligibility Questionnaire, Food Diary, and Daily Symptoms Questionnaire

THE EFFECTS OF A PROBIOTIC SUPPLEMENT ON
SYMPTOMS OF ADHD AND ANXIETY

Researchers:
Dr. Susan Potter, Research supervisor, email: susan.potter@acadiau.ca, tel: (902) 585-1220
Dr. Mark Johnston, Physician researcher, email: markerj1@me.com, tel: (902) 679-0536

You and your child have been invited to participate in a study examining the effects of probiotics on the symptoms of attention deficit-hyperactivity disorder (ADHD), anxiety, and associated physiological and psychological factors, as described in the project information sheet. Please review the information sheet before consenting to participate.

This consent form is for the initial online questionnaire (described in section 4 on the information sheet) and one-week information collection period (food diary and daily questions about symptoms described in section 6 on the information sheet) only.

These questionnaires and food diary will help the researchers determine if your child is a good candidate for the study and provide them with useful information if your child meets the eligibility criteria and you and your child decide to participate.

By checking the box below you are indicating that you are the legal parent or guardian of your child and that you have signing authority for your child, that you have read the information sheet, that you understand the nature of the study and what is required of you and your child, and you are providing your free and informed consent to participate in the initial screening questionnaire for this study. By consenting to participate, you are not waiving any of your legal rights by consenting to participate in this study. Your participation in this research project is greatly appreciated.

Contact information collected during this study will be used only for the purpose of this study.

☐ By clicking “SUBMIT”, I acknowledge that I have read and understand the above information and hereby consent to participate in the initial screening portion of this study. I am free to discontinue my participation at any time.

SUBMIT

Note: if you encounter any difficulties while completing this questionnaire, please click the back button on your browser and try clicking “next” again. If you continue to encounter difficulties please email the researcher.
Appendix C

**Online Questionnaire (Demographic and Screening Questions)**

Please provide all of the following information:

Your Name: ________________________________________________

Relationship to child: _______________________________

Address: __________________________________________________

E-mail Address: ________________________________________

Phone: Home: __________________________ / Cell: ______________________

Child’s Name: __________________________________________

Child’s Age: _________

Child’s Gender: □ Male □ Female

**NEXT**

Is your child currently taking any medication for inattentive and/or hyperactive/impulsivity symptoms?

□ Yes

□ No

If you answered yes to the above question, please specify which medication(s) your child is taking, the dose, and how long they have been taking it:

Is your child currently taking any medication for anxiety symptoms?

□ Yes

□ No

If you answered yes to the above question, please specify which medication(s) your child is taking and how long they have been taking it:

_______________________________________________________________________

_______________________________________________________________________

If your child is currently taking any other medications (for symptoms other than those associated with attention deficit-hyperactivity disorder or anxiety disorders) please list them here:

_______________________________________________________________________

Does your child have any diagnosed illnesses?

□ Yes

□ No

If yes, please specify the illness/illnesses:

Has your child been tested for any thyroid diseases?

□ Yes

□ No
If yes, please specify any diagnosis:
___________________________________________________

Has your child ever had his or her cortisol levels tested?
☐ Yes
☐ No

If yes, please specify the results:
______________________________________________________

Has your child experienced any yeast- or fungal-infections in the past two years?
(including skin rashes/fungal infections such as athlete’s foot or Candidiasis vaginal yeast infection, thrush of the mouth/throat, etc.)
☐ Yes
☐ No

If yes, please specify:
_______________________________________________________________

Does your child have any diagnosed psychiatric, developmental, or neurological conditions?
☐ Yes
☐ No

If yes, please specify:
_______________________________________________________________

Does your child currently attend sessions with a mental health professional?
☐ Yes
☐ No

Please answer the following questions with respect to your child’s behavior. Indicate how much the following statements apply to your child during the past 6 months, using the following scale:
1 = “does not apply to my child at all” or “never occurs”
2 = “rarely applies to my child” or “occurs infrequently”
3 = “applies to my child somewhat” or “sometimes occurs”
4 = “often applies to my child” or “occurs frequently”
5 = “always applies to my child” or “is always true”

<table>
<thead>
<tr>
<th>Inattention Symptoms:</th>
<th>1 (never)</th>
<th>2 (rarely)</th>
<th>3 (sometimes)</th>
<th>4 (often)</th>
<th>5 (always)</th>
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<tbody>
<tr>
<td>Has difficulty giving close attention to details in schoolwork or other activities</td>
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<tr>
<td>Has difficulty sustaining attention in chores or play</td>
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<td>Does not appear to listen when spoken to</td>
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<td>Cannot follow through on given instructions (but instructions are understood)</td>
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<tr>
<td>Cannot organize his or her own tasks or activities</td>
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<tr>
<td>Avoids or dislikes activities that require sustained attention</td>
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<td>Often loses track of his or her possessions (pencils, schoolwork, toys, etc.)</td>
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<td>Easily distracted by other things in his or her environment</td>
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<tr>
<td>Is forgetful in his or her everyday activities</td>
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</table>
Indicate how much the following statements apply to your child during the past 6 months, using the following scale:
1 = “does not apply to my child at all” or “never occurs”
2 = “rarely applies to my child” or “occurs infrequently”
3 = “applies to my child somewhat” or “sometimes occurs”
4 = “often applies to my child” or “occurs frequently”
5 = “always applies to my child” or “is always true”

<table>
<thead>
<tr>
<th>Hyperactive/Impulsive Symptoms</th>
<th>1 (never)</th>
<th>2 (rarely)</th>
<th>3 (sometimes)</th>
<th>4 (often)</th>
<th>5 (always)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fidgets or squirms in his or her seat; has difficulty sitting still</td>
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<td>Has difficulty staying in his or her seat in situations where it is expected</td>
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<td>Is overly active, running and climbing when not appropriate for the situation</td>
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<td>Difficulty engaging in leisure activities quietly</td>
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<td>Talks excessively</td>
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<td>Could be considered “on the go” or “driven by a motor”</td>
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<td>Has difficulty waiting for his or her turn when in a group</td>
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<td>Interrupts other children’s games or intrudes into other’s activities inappropriately</td>
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<td>Frequently answers questions before the speaker has finished saying the question</td>
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</table>
Indicate how much the following statements apply to your child during the past 6 months, using the following scale:
1 = “does not apply to my child at all” or “never occurs”
2 = “rarely applies to my child” or “occurs infrequently”
3 = “applies to my child somewhat” or “sometimes occurs”
4 = “often applies to my child” or “occurs frequently”
5 = “always applies to my child” or “is always true”

<table>
<thead>
<tr>
<th>Anxiety Symptoms:</th>
<th>1 (never)</th>
<th>2 (rarely)</th>
<th>3 (sometimes)</th>
<th>4 (often)</th>
<th>5 (always)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is more shy and anxious than other children his or her age</td>
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<tr>
<td>Is more worried than other children his or her age</td>
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<tr>
<td>Is afraid of many more things than other children his or her age.</td>
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<tr>
<td>Worries that something terrible is going to happen to him/herself or his/her family</td>
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<td>Is afraid to sleep away from home (e.g., sleep-overs at his or her friends’ houses)</td>
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Thank you for your time and interest in this research project. A researcher will be in contact with you within approximately one week to discuss your potential participation in this study, and to set up an initial meeting for you and your child.

If any of these questions have left you concerned about your child’s behavior and you would like to speak a professional, please contact one of the following:

- Your child’s therapist, if applicable;
- Halifax Community Mental Health, Tel: (902) 422-1611
- Annapolis Valley Health, Health Education, Tel: 1-877-365-1735
- Mental Health, King’s County, Child & Youth Program, Tel: (902) 679-2873

Once you click the SUBMIT button at the bottom of the page, you will be redirected to the Probiotic Study homepage.
Appendix D

Consent Form – Intervention Phase

THE EFFECTS OF A PROBIOTIC SUPPLEMENT ON SYMPTOMS OF ADHD AND ANXIETY IN CHILDREN

Researchers
Dr. Susan Potter, Research supervisor, email: susan.potter@acadiau.ca, tel: (902) 585-1220
Dr. Mark Johnston, Physician researcher, email: markerj1@me.com, tel: (902) 679-0536

You and your child have been invited to participate in a study examining the effects of probiotics on the symptoms of attention deficit-hyperactivity disorder (ADHD), anxiety, and associated physiological and psychological factors, as described in the project information sheet. Please review the information sheet before consenting to participate. Section 6 of the information sheet describes what you and your child will be asked to do at each step in the study. Contact information collected during this study will be used only for the purpose of this study.

By signing below you are indicating that:
- you are the legal parent or guardian of the child named on the form and that you have signing authority for the child
- you have read the information sheet and understand the nature of the study and what is required of you and your child
- you have explained the study to your child and he or she has agreed to participate
- you are providing your free and informed consent to participate in this study with your child
- you are not waiving any of your legal rights by consenting to participate in this study

Please check the box below if you consent for your child’s teacher to know of their participation in the study and comment on any change in your child’s ADHD and/or anxiety symptoms. Your child's name will appear on the teacher questionnaire until it has been received by the researcher, at which point it will be changed to your child's code number.

☐ I consent for my child’s teacher to know of his or her participation in this study.

☐ I consent for my child’s teacher to answer questions about my child’s ADHD and/or anxiety symptoms and acknowledge that the teacher’s answers will not be shared with me.

We may carry out similar studies in the future. Please check the box below if you are willing to be contacted in the future about participating in other similar studies. Your time, participation, and contribution to this research project and science in general are greatly appreciated. Any contact information disclosed below will be used only for the
purpose of informing you of our future studies. Checking this box does not mean you have to participate in these studies, you will simply be notified.

☐ I am willing to be contacted about participating in other similar studies in the future.

Name: ___________________ Email address: _________________ Phone number: __________

Please indicate why you and your child are choosing to participate in this study:

_________________________________________________________________________

Name of Child: __________________________________________

Name of Parent: _________________________________________

Signature of Parent: ___________________________ Date:

____________________

Signature of Researcher: _______________________ Date:

____________________
Appendix E

Daily Questionnaire

To be Completed Each Evening By the Child

Bowel Health Questions for Kids

1. Did your tummy feel okay today?
   - Yes
   - No

2. How much did your tummy hurt or feel uncomfortable today?
   - 0. No hurt
   - 1. Hurts a little bit
   - 2. Hurts a little more
   - 3. Hurts even more
   - 4. Hurts a whole lot
   - 5. Hurts worse

3. Was your pooping okay today?
   - Yes
   - No

4. How many times did you poop today?
   - I did not poop yet today.
   - Once
   - Twice
   - Three Times
5. What was your poop like today? Choose from the chart.
   - Type 1
   - Type 2
   - Type 3
   - Type 4
   - Type 5
   - Type 6
   - Type 7
   - I did not poop yet today.

6. Did you have to push really hard to get your poop out at all today?
   - Yes
   - No
   - I did not poop yet today.

7. Did you ever feel like you had to rush to the bathroom to poop today?
   - Yes
   - No

8. Did you miss any school or playtime today because of problems with your tummy or pooping?
   - Yes
   - No

Feelings Questions for Kids

1. How worried or anxious were you overall today? (Please select one)
   - 1- No Anxiety
   - 2

   ![Feelings Faces]
2. How easy or hard was it for you to sit still when you were supposed to today?

- 1 - Very easy
- 2 - A little bit easy
- 3 - In the middle, between easy and hard
- 4 - A little bit hard
- 5 - Very hard

3. How easy or hard was it for you to pay attention and not be distracted today?

- 1 - Very easy
- 2 - A little bit easy
- 3 - In the middle, between easy and hard
- 4 - A little bit hard
- 5 - Very hard

Good job! You finished your questions for today!
Appendix F

Daily Questionnaires
To be Completed Each Evening By the Parent/Guardian

Feelings Questions for Parent/Guardian of Child
The following questions are to be completed by the parent/guardian.

1. How worried or anxious was your child overall today? (Please select one)
   - 1 - No Anxiety
   - 2
   - 3
   - 4
   - 5 - Extreme Anxiety

2. How easy or hard was it for your child to sit still when he/she was supposed to today?
   - 1 - Very easy
   - 2 - A little bit easy
   - 3 - In the middle, between easy and hard
   - 4 - A little bit hard
   - 5 - Very hard

3. How easy or hard was it for your child to pay attention and not be distracted today?
   - 1 - Very easy
   - 2 - A little bit easy
   - 3 - In the middle, between easy and hard
   - 4 - A little bit hard
   - 5 - Very hard

4. Did your child attend any psychotherapy or counselling sessions today?
   - Yes
   - No

Question 4 continued: If applicable describe your child's session...

Thank-you for completing your daily questionnaire.
Appendix G

Child Daily Food Diary

***to be completed for the first seven days only***

<table>
<thead>
<tr>
<th>Code #:</th>
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<tbody>
<tr>
<td>Date:</td>
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<table>
<thead>
<tr>
<th>Time of day</th>
<th>What did you eat and drink?</th>
<th>How much did you have?</th>
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</table>
Appendix H

Participant Instructions
Weeks 2 – 5: Intervention Phase 1 and 10 - 13: Intervention Phase 2

THE EFFECTS OF A PROBIOTIC SUPPLEMENT ON
SYMPTOMS OF ADHD AND ANXIETY IN CHILDREN
Acadia University

Researchers:
Dr. Susan Potter, Research supervisor, email: susan.potter@acadiau.ca, tel: (902) 585-1220
Dr. Mark Johnston, Physician researcher, email: markerj1@me.com, tel: (902) 679-0536

The packets that you receive for the first and second four week intervention phases of the study will contain either probiotics (beneficial bacteria) or placebo (it looks and tastes the same but has no health benefits). You will not be told whether your child is taking probiotics or placebo. He or she will take the same one for four weeks then switch and take the other for four weeks, with four weeks in between, a “wash-out period”, when they won’t take any -- if your child receives placebo for the first phase, he or she will receive probiotics for the second phase; or vice versa. If you or your child wishes to know which one they got first and second, we can tell you after the study is completed.

Your child should consume one packet of the probiotic or placebo powder each day, mixed thoroughly in a glass of milk or sprinkled on/mixed in a bowl of ice-cream. Try to have your child take the powder at approximately the same time each day. If you forget to give it or they are unable to take it at the same time one day, please get them to take it as soon as possible and resume the normal schedule the following day. Please consider the dietary instructions on the following page.

You will receive daily email-reminders about taking the probiotics as well as emails from the researcher on day 3 and day 6 (Appendix S) to check in and see how your child is doing. If you or your child has any questions or concerns, please feel free to reply to these emails and a researcher will answer you as soon as possible. You may also contact the researcher (by the email listed above) anytime throughout the study.

In approximately two weeks, you will be contacted by the researcher in order to confirm a date and time for your second meeting.

Near the end of the last week of each phase (week 5, 9 and 13), you will collect another saliva sample from your child, first thing in the morning. It should be done immediately after your child wakes up, before eating anything. Follow the instructions in the saliva collection kit. It is important that you store your child’s saliva sample in a freezer. Please bring the saliva samples to your meeting with the researcher.
Following Phase 1, there will be a four-week “wash-out” period (during weeks 6, 7, 8 and 9). During this time your child won’t take any of the powder. However, you should both continue to complete the weekly questionnaires so we can monitor any changes during this four weeks.

Note: It is important that you contact the researcher immediately should your child begin taking antibiotics, any new medication (including antacids), or develop a new medical condition.

Foods that your child should avoid for the duration of the study:

- Probiotic Yogurt (including probiotic frozen yogurt or frozen drinks/smoothies)
  - Some brands are okay because they don’t contain probiotics. During the study period it is okay to eat the following kinds of yogurt: (list to be inserted)
- Kefir
- Kimchi (kimchee, kim chee or gimchi)
- Unpasteurized/uncooked tempeh
- Unpasteurized/uncooked miso
- Unpasteurized, fermented sauerkraut
- Sour cream
- Cottage cheese
- Most uncooked cheese (cheddar, gouda, etc.)
  - Cheese produced by Arla and Saputo may be consumed during the study because they do not contain probiotics, and processed cheese slices are also acceptable
- Unpasteurized soy sauce/tamari
- Any other foods that contain probiotics

If you have any questions or concerns at anytime throughout the duration of the study, please feel free to contact the researcher.
Appendix I

Saliva Sample Collection

- You will help your child collect his or her first saliva sample one morning shortly after meeting with the researchers, BEFORE beginning to take the probiotic or placebo powder. Instructions for saliva sample collection are as follows:
  - **First sample (Labeled “#1”)**: You should collect your child’s first baseline saliva sample as soon as possible after your first meeting with the researchers, before beginning to take the probiotics or placebo. Do this first thing in the morning immediately after your child wakes up (have the kit ready the night before, in the child’s bedroom). They should not eat or drink anything until after you have collected the saliva sample. Write the date and time on the label. Follow the instructions with the kit provided. If his or her mouth is too dry, get him/her to try to imagine biting into a lemon or eating a favourite food. After collecting the sample, immediately place it in your freezer (deep freezer if you have one). Bring the saliva sample to your first meeting with the researcher.
  - **Second sample (Labeled “#2”)**: Your child’s second saliva sample should be collected near the end of the first probiotic or placebo phase (do it during the last week, prior to your appointment with the researcher). As before, collect the sample first thing in the morning. Write the date on the label. Place the sample in your freezer.
  - **Third sample (Labeled “#3”)**: Collect this as described above, at the end of your child’s four-week wash-out period (i.e., the four weeks during which they do not take the powder). Write the date and time on the label.
  - **Fourth sample (Labeled “#4”)**: Collect this as described above, at the end of your child’s last four weeks in the study in which they take probiotics or placebo powder (during the last week, before your final meeting with the researcher). Write the date and time on the label.
  - Bring the saliva samples to your next meeting with the researcher. It is okay if the samples cannot be refrigerated for an hour or two, but try to keep them frozen for as long as possible.