The effects of probiotic supplementation on endurance athletes: Influences on gut dysfunction, microbiome, and gut discomfort

Sarah Lennon

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The effects of probiotic supplementation on endurance athletes: Influences on gut dysfunction, microbiome, and gut discomfort

by

Sarah Lennon

A Thesis Submitted in Partial Fulfillment of the Requirements of the Degree of Master of Science

Major: Nutrition Science

The University of Memphis

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First and foremost, I want to thank and acknowledge my boyfriend, Dylan, for supporting and encouraging me throughout this entire process. Also, I would like to thank my friend Thomas Lackie who made invaluable contributions to this research study. One final acknowledgement is towards the members of my thesis committee for their help.
ABSTRACT

This study was a randomized placebo-controlled, double-blinded crossover study elucidating the effects of the probiotic on endurance athletes who experienced GI discomfort and dysfunction. Athletes were recruited to participate if they met multiple criteria involving their running habits. Multiple metrics were utilized to analyze the degree to which the probiotic altered the microbiome and reduced GI Discomfort and Dysfunction within endurance athletes. Markers of intestinal permeability collected were Zonulin, IFABP, and LBP. GI Discomfort was measured via participant self-reporting of symptoms as well as through the Borg Scale. Microbiome changes were measured looking at alpha and beta diversity changes and analysis of the genera distributions. The probiotic had no significant changes associated with any metric. Therefore, it is concluded within this study that there is no therapeutic benefit to probiotic for endurance athletes to alleviate GI related issues or alter the gut microbiome.
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<table>
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<th>Description</th>
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<tr>
<td>SCFA</td>
<td>Short Chain Fatty Acid</td>
</tr>
<tr>
<td>LBP</td>
<td>Lipopolysaccharide Binding Protein</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>IFABP</td>
<td>Intestinal Fatty Acid Binding Protein</td>
</tr>
<tr>
<td>BPM</td>
<td>Beats per minute</td>
</tr>
<tr>
<td>GALT</td>
<td>Gut Associated Lymphoid Tissue</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-Like Receptor</td>
</tr>
<tr>
<td>NLR</td>
<td>NOD-like receptor</td>
</tr>
<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular pattern</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable Bowel Disease</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
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<tr>
<td>URTI</td>
<td>Upper respiratory tract infection</td>
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Background

Humans have developed a very complex, highly specialized gastrointestinal (GI) system for the purpose of digestion and absorption. It is continuously exposed to foreign antigens produced by food or microbiota and comprised of the largest compartment of the immune system. As such, due to this constant exposure to foreign infiltrates the immune response needs to react appropriately to each threat. However, it must simultaneously regulate its response to the symbiotic relationship formed with the intestinal microbiota. Beyond foreign antigens, factors such as diet and exercise also stress this system. Diets have been shown to alter the composition of the microbiome. Exercise has been shown to elevate permeability via tissue damage. Therefore, discerning the interplay of these influences from foreign antigens to exercise is critical to our understanding of the gut’s influence on both human health as a whole and the immune response specific to the gut.

The immune response within the gut is tightly regulated as it needs to be functional against pathogens, while also being nonreactive to the commensal microbiota residing within the intestinal lumen. One example of this process occurs through gut-associated lymphoid tissue (GALT), which helps mediate the inflammatory response within the intestines. Numerous studies have shown that the composition of the intestinal microbiota affects the composition of GALT; thereby, influencing the lymphoid tissue response. Thus, it can be stated that the relationship between the GI microbiota and the intestinal immune system is multifaceted and complex as there needs to be a tempered and appropriate response to both the health promoting and pathological bacteria existing within the intestines. This response is moderated by signaling cascades generated in response to pathogen-associated molecular patterns (e.g.,
lipopolysaccharides, peptidoglycan, flagellin, and microbial nucleic acids). Through various Toll-like receptors (TLRs) that send stimulatory signals to interplay with NOD-like receptors (NLRs) to respond appropriately and avoid both under and over stimulation by intestinal epithelial cells to bacterial PAMPs. Therefore, this response is critical for intestinal and host health, and as the microbiota can affect and alter the response, the interactions between diet, exercise, and other factors influencing the microbiome are critical junctures to elucidate this complex relationship. 

The intestinal microbiota represents one of the most diverse ecosystems in nature. The number and type of microbes vary along the GI tract, but most microbes are located within the large intestine. Here they assist with the fermentation of undigested food components and fecal bulk. Some of the most commonly found gut bacteria in adults include Bifidobacterium, Lactobacillus, Bacteroides, Clostridium, Escherichia, Streptococcus, and Ruminococcus. There is a high degree of variability among the composition of microbes between individuals; however, despite the considerable variation, basic metabolic activities between individuals are highly conserved.

Recent research has shown that the gut microbiome’s role in human health is implicated in the progression and pathophysiology of many diseases. It has been demonstrated to have a role in metabolism and obesity. It has also been shown to impact cancer pathogenesis, chronic GI non-communicable diseases, functional gastrointestinal disorders, cardiovascular disease, and various autoimmune conditions. Also, alterations in the gut microbiota have been linked to neurodegenerative diseases and mood disturbance.
Microbiome

Over the last several years, there has been an explosion of information regarding the role of the gut microbiota and human health. The gut microbiome has been shown to regulate many intestinal functions, including barrier maintenance, immune education, and inflammatory status. Due to our improved understanding of the role of various microbes in human health, probiotics are increasingly utilized as a complementary therapy for various pathological conditions. Probiotics are living organisms consumed through food or supplementation and are beneficial to human health. The advantage of using probiotics is that they have a good safety profile and fewer side effects compared to many traditional therapies. The use of probiotics has been shown to be especially beneficial in intestinal disorders such as irritable bowel disease (IBS) and colitis, possibly through increased intestinal short-chain fatty acids (SCFA) production and improved intestinal barrier function.

With the development of new molecular techniques and bioinformatics, research has explored the intestinal microbiota’s role in different physiological processes. This has allowed researchers to examine the role of intestinal microbes beyond their role in the digestive process. The gut microbiome has many functions, including constituting the intestinal barrier, stimulating intestinal epithelial cell regeneration, mucus production, and the nourishment of the mucosa by producing SCFAs. The gut microbiome also plays a role in the maturation of the immune system. It stimulates the innate immune system in the early stages of life, causing intestinal-related lymphoid tissue maturity. Under normal physiological conditions, the gut microbiota supports and strengthens the intestinal barrier function and gut immune system across the lifespan.
Short-Chain Fatty Acids

SCFAs are the main products of the anaerobic fermentation of indigestible polysaccharides like dietary fiber and starch and include acetate, butyrate, and propionate etc. They are produced by the microbiome in the large intestine. The amount of SCFAs produced in the gut can range between 500-600 mmol per day. The amount of fiber in the diet and microbiome composition can influence the quantity produced. Acetate, propionate, and butyrate are also produced from amino acid metabolism, but less than 1% of SCFAs are produced this way. SCFAs have been shown to improve gut health in many different ways including maintenance of intestinal barrier integrity, mucus production and protection against inflammation.

Factors Affecting the Microbiome

The gut microbiome is ever-changing throughout the life cycle. The microbiome is influenced by several factors, including feeding, the aging process, diet composition, and geography. Other factors that can influence the microbiome include NSAIDs, probiotic supplements, drug and alcohol abuse, GI surgery, Celiac disease, IBS, antibiotic usage, and immunosuppression.

It was previously thought that the gut microbiome is sterile at birth, and it is only recently that there is work that suggests otherwise. It has been hypothesized that swallowing amniotic fluid and its bacteria start the colonization process. The microbiome is then further developed during the birthing process. The mode of delivery has a major impact on the infant’s microbiota composition. It is currently believed that gut colonization can be enhanced by natural birth.
Babies delivered naturally have proven to have higher gut bacterial counts at one month of age than their cesarean delivered counterparts. The development of the gut microbiome continues as the infant ages. Multiple differences have been found between breast-fed infants and formula-fed infants. The microbiome of breastfed infants is significantly enriched in >600 species of bacteria including *Bifidobacterium breve*, *adolescentis*, *longum, bifidum*, and *dentium* in comparison to those who are not breastfed. Another significant change in the microbiome happens when infants start to consume solid foods. This initial shift in diet from breastfeeding/formula is to utilize plant-derived glycans before the introduction of solid food. Then, after the introduction of solid foods, the microbiome composition begins to resemble that of an adult. The microbiome goes from being dominated by *Bifidobacterium* to *Bacteroidetes* and *Firmicutes*. This typically remains relatively stable throughout adulthood, assuming no long-term dietary changes or repeated antibiotic use.

Factors affecting the gut microbial composition include diet, medication, and lifestyle factors such as level or activity and stress. Non-steroidal anti-inflammatory drugs (NSAIDs), for example, are widely used for conditions such as osteoarthritis, rheumatoid arthritis, fevers, and various pain symptoms. NSAID users often have intestinal conditions such as inflammation, mucosal erosion, ulceration, and increased mucosal permeability have been seen. NSAIDs have also been shown to affect the composition of the gut microbiome. Consumption of aspirin has been shown to raise the abundance of *Prevotella*, *Bacteroides*, *Ruminococcaceae*, and *Barnesiella*. Individuals taking ibuprofen were shown to have an increased abundance of bacteria from families *Propionibacteriaceae*, *Puniceicoccaceae*, *Pseudomonadaceae*, and *Rikenellaceae* in comparison to individuals who were non-users or regularly took naproxen.
Diet has been shown to be a major determinant of the composition, abundance, and diversity of the microbiota. Dietary components such as fiber, probiotics, and polyphenols have all been shown to have positive effects on the gut. In comparison, a western-style diets composed of increased levels of sugar and fat with a low intake of dietary fiber can irreversibly reduce microbial diversity and even eradicate specific species from the digestive system as a whole. Because diet heavily impacts the microbiome, individuals can typically be placed into two groups based on their nutritional habits. Individuals who consume primarily high complex carbohydrates typically have more *Prevotella* bacteria. In comparison, people who consume a higher fat, higher protein diet have more *Bacteroides*.

These diet-derived changes can possibly result in dysfunction, which in turn can lead to the increases in the development of chronic inflammatory diseases, including IBS, colorectal cancer, allergies, and autoimmune disorders. It is hypothesized that these pathologies can partially be prevented by including more dietary fiber.

Alcohol is another common disruptor of the microbiota. Due to its absorption and secondary metabolism within the GI tract, it is a factor that could impact the gut. Alcohol use has been associated with changes in the colonic bacterial composition in a subset of alcoholics in comparison to healthy controls. This alteration has been shown to be correlated with high levels of serum endotoxin. However, not all alcohol has a negative effect. Red wine consumption has been shown to modulate the growth of select bacteria in humans, specifically an increase in *Proteobacteria, fusobacteria, firmicutes, and bacteroidetes*. It’s believed that the polyphenols in the wine provide a prebiotic effect leading to these changes.
Since the discovery of penicillin, antibiotics have been a great therapy in the fight against bacterial infection. However, this life saving medication also affects the bacteria that reside in the intestinal lumen. Antibiotic use, whether orally or intravenously, has been shown to reduce the number of bacteria, prevalence, and in some individuals the elimination of certain bacterial communities as a whole. Each individual antibiotic has its own impact and alteration on the gut microbiome. On average, though, most microbiota changes return to normal 2-4 weeks after discontinuation of the antibiotic.

**Markers of Permeability**

Intestinal dysfunction and gut permeability can be monitored by the appearance of various gut associated molecules including intestinal fatty acid binding protein (IFABP), lipopolysaccharide-binding protein (LBP) and Zonulin.

**FABP**

There are three different types of fatty acid-binding proteins found in the intestine. Intestinal fatty acid-binding protein (IFABP) is found in the enterocytes of the jejunum and the colon, while liver-type FABP is found throughout the intestine as well as in the liver and kidney. Ileal bile acid-binding protein (IBABP) is found only in the ileum. IFABP is a cytosolic protein that plays a vital role in the cellular uptake and metabolism of fatty acids in enterocytes and only small amounts are found in the blood under normal physiological conditions. Elevated levels of IFABP have been suggested as a marker of intestinal permeability. Increased levels of IFABP have been shown to be statistically significant with increased levels of enterocyte damage.
Adriaanse et al. (2013) showed elevated levels of IFABP within individuals who suffer from celiac disease as a marker of tissue damage.\(^{35}\)

**Lipopolysaccharide Binding Protein**

Lipopolysaccharide binding protein (LBP) is an acute-phase protein produced by hepatocytes and released into the bloodstream. LBP binds to bacterial lipopolysaccharide (LPS), which translocated across the intestinal barrier from the intestine. As LPS levels increase in response to endotoxins and gut inflammation, LBP increases as well, and is therefore considered a marker of endotoxemia as well as a biomarker for intestinal permeability.\(^{36}\) This is due, in large part, to the fact that when intestinal tissues are damaged, LPS will be bound to LBP, which will, in turn, instigate the inflammatory cascade as LPS bound to LBP activates macrophages when bound to TLR4 with CD-14 as a cofactor to release cytokines.\(^{37}\)

**Zonulin**

Zonulin comprises both structural and functional related proteins that are described as zonulin family peptides. Zonulin is an acute phase reaction protein, and it functions in intestinal permeability by decreasing the stability of tight junctions.\(^{38}\) It is also believed that zonulin helps protect against microorganism colonization.\(^{39}\) When Zonulin is deregulated in genetically susceptible individuals, autoimmune, inflammatory, and neoplastic disorders can occur.\(^{40}\) When exposed to bacteria or other triggers that cause autoimmune responses by the immune system, zonulin is released into the serum, which is hypothesized to be a mechanism by which microorganisms are flushed out of the gut (the exact mechanism is unknown), but also it functions to begin the inflammatory cascade in response to these triggers. Therefore, zonulin within serum can be utilized as a measure of permeability as its presence in the serum indicates both a release from tight junctions but also a measure of an inflammatory response.\(^{41}\)
**Probiotics**

Probiotics are living microorganisms that present benefits to hosts when correctly administered. Probiotics can be obtained via fermented foods or microbes through a supplement. They can potentially provide various health benefits to the individual, including normalizing the perturbed microbiota and intestinal motility, competitively excluding pathogens, and increasing SCFA production. Probiotics contain microorganisms that are similar to the bacteria found in the gut microbiome. Commonly probiotics contain the bacteria *Bifidobacterium* and *Lactobacillus*. Lactobacilli species are used in probiotics since they have the capability to adhere to intestinal surfaces, have a high tolerance for acid and bile, can withstand low pH, gastric juice, can inhibit potentially pathogenic species and resist antibiotics. Strains of *Bifidobacterium* are also used because of their resistance mechanisms to bile salts. This is important because the positive effects of probiotic bacteria need to be generated in the presence of bile salts.

It is worth noting that the probiotic market is poorly regulated unless specific disease-related claims are made. Probiotics are also trademarked by brand and not bacterial strain. This means that formulations can change over time, which can have a significant impact on efficacy.

Probiotics are known to regulate intestinal homeostasis, interfere with pathogens’ ability to colonize the mucosa, modulate immune responses and assist with the stabilization and maintenance of the gastric intestinal barrier functions. Unlike other medications and supplements, many probiotics do not have one clear mechanism of action. Given that there are so many strains and different formulations, there is no one clear answer for all probiotics. This is due, in large part, to the fact that probiotics as a whole have a myriad of unique effects due to
their capability to express particular surface molecules or secrete products that can interact with components of epithelial cells that form the epithelial barrier or even the cells in the immune system that are underneath this barrier. These properties can further depend on if the probiotic organism is viable or dead.

GI and Running

In endurance sports, gastrointestinal (GI) discomfort is widely reported during prolonged endurance exercise, with 27% of recreational runners reporting GI discomfort characterized by symptoms such as belching, bloating, flatulence, abdominal cramping, side stitch, nausea, vomiting, diarrhea, and the urge to defecate during exercise. This condition can be detrimental to exercise performance in both recreational and elite runners. The cause of the discomfort is multifactorial and includes a change in blood flow as blood is shunted from the viscera to skeletal muscle and the heart. This results in hypoxia and can impact the permeability of the intestinal barrier by reducing epithelial tight junctions. Loss of epithelial integrity results in translocation of bacterial products, including lipopolysaccharide, and increasing release of systemic inflammatory molecules. This can possibly lead to an increased susceptibility to infectious and autoimmune diseases because of the absorption of pathogens/toxins into tissues and the bloodstream. Recent research has begun to look at whether probiotic supplementation positively impacts the reduction of GI symptoms and improves aerobic performance.

Probiotic supplementation is believed to increase the GI tract’s resilience, which has particular interest to athletes, more specifically those involved in prolonged endurance events. These athletes often have the greatest occurrence of GI problems that will lead them to stop or will
significantly hinder performance. It is known that prolonged or strenuous exercise may increase key phosphorylation enzymes. These will disrupt tight junction proteins and may provoke immune responses and endotoxin-associated symptoms characteristic of GI complaints endurance athletes often complain about. 7

Probiotics and Exercise

As many of the symptoms of exercise-induced GI discomfort resembles that of IBS and colitis, probiotics are increasingly tested for the improvement of exercise-induced GI symptoms. Regular use of probiotics can alter both the abundance and structure of the microbial community and also affect immune responses in healthy as well as trained individual. 7 As the effects of probiotics differ based on differences in the mechanism of action of the specific strain used, different strains and combinations of strains are being examined for therapeutic function. Recent research has begun to look at how probiotics have a role in modulating GI discomfort in athletes. The 2011 study by West et al. looks at how the probiotic L. Fermentum used daily reduced symptoms of gastrointestinal and upper respiratory tract illnesses by modulating commensal microflora. Overall looking at all participants, this study reported an increase in the number and duration of low-grade self-reported gastrointestinal symptoms. However, when looking at men alone, there was a slight decrease in the self-reported severity score of GI symptoms in participants on the probiotic compared to the placebo. 55

West et al. completed a similar study in 2012 with active individuals who took the multistrain probiotic Gut Balance™. This probiotic contained Lactobacillus paracasei subsp paracasei (L. casei 431®), Bifidobacterium animalis ssp lactis (BB-12®), Lactobacillus acidophilus (La-5®), Lactobacillus rhamnosus (LGG®), two prebiotics (raftiline and raftilose), and bovine whey
derived lactoferrin and immunoglobulins with acacia gum. In this case, they found that the probiotic has little to no effect on mucosal immunity or gut permeability. 56

Previous research has also examined the effects of probiotics on respiratory infections and gastrointestinal symptoms. West et al. recruited 141 marathon runners to complete a randomized, double-blind intervention study where they received a probiotic (Lactobacillus rhamnosus GG) or a placebo for a three-month training period. At the end of the training period, participants completed a marathon. The study found both no numerical differences and effects on the incidence of respiratory symptoms in runners. It did, however, shorten the duration of GI symptoms. 56

The 2017 study by Karhu et al. investigated running-induced changes and intestinal permeability and markers of GI function to investigate whether they were correlated with gastrointestinal symptoms. This study enrolled 17 active runners. Participants did a 90-minute treadmill run at 80% of their best 10k race speed. These runners were both symptomatic and asymptomatic. Intestinal permeability markers including serum intestinal fatty acid-binding protein, zonulin, bacterial lipopolysaccharide and fecal calprotectin were measured both prior to the treadmill test and after completing the running test. 58 The study concluded a significant increase in intestinal permeability and serum intestinal fatty acid-binding protein, but there was no difference between symptomatic and asymptomatic runners. The study suggested that intestinal permeability changes alone do not account for GI symptom development while running. 58

A multi-strain probiotic consisting of Bifidobacterium bifidum W23, Bifidobacterium lactis W51, Enterococcus faecium W54, Lactobacillus acidophilus W22, Lactobacillus brevis W63, and Lactococcus lactis W58 was used to explore the effects of supplementation on GI permeability in
endurance-trained men (probiotic group n=11, placebo group n=12). The study concluded that zonulin decreased with supplementation from a value slightly above normal into normal ranges. Zonulin was also significantly lower after 14 weeks with probiotics compared to the placebo. Carbonyl proteins increased significantly between pre- to post-exercise both groups at baseline. After 14 weeks of treatment, concentrations were slightly lower with probiotics. There was no impact on α1-antitrypsin. In conclusion, they concluded that probiotic treatment decreases Zonulin in feces indicating that probiotic supplementation can improve intestinal barrier dysfunction. 54

A multispecies probiotic (L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. fermentum, B. lactis, B. breve, B. bifidum, and S. thermophilus) was also utilized to determine whether probiotic supplements reduce gastrointestinal permeability and systemic levels of endotoxin and inflammation following exercise in the heat and increase run time to fatigue while blunting the rise in core temperature and gastrointestinal disturbances. This was a randomized, double-blind crossover trial with ten male runners. The probiotic supplementation significantly increased run-time-to-fatigue, with average core temperature during exercise remaining similar between both the initial trial and the test trial. Plasma concentrations of IL-6, IL-10, and IL-1ra were all increased after exercise, but there was no significant difference between the two trials. In addition, they found the ratio of lactulose to rhamnose, an indicator of gut permeability, to be lower when compared to the placebo treatment. However, this result was not statistically significant (p= 0.35). They concluded that although run time was longer following probiotic supplementation, the circulating concentrations of pyrogenic cytokines and core temperature were similar. 59
Pugh et al. (2019) completed a 4-week probiotic supplementation study also utilizing a multi-strain probiotic consisting of 25 billion CFU \textit{Lactobacillus acidophilus} (CUL60 and CUL21), \textit{Bifidobacterium bifidum} (CUL20), and \textit{Bifidobacterium animalis} subs p. \textit{Lactis} (CUL34). They examined probiotic supplementation on gastrointestinal symptoms, circulatory markers of GI permeability damage, and markers of the immune response during a marathon race. A total of 24 runners participated in the study, 20 male and four female participants. Before starting supplementation, participants visited the lab four weeks before the initial marathon and completed a gastrointestinal symptoms rating scale. This was used to assess baseline GI symptoms. Participants then completed an incremental running test to determine lactate threshold and peak oxygen uptake. After baseline testing, participants underwent a 28-day period of supplementation consuming either the probiotic or the placebo. During the supplementation, participants were also instructed to avoid probiotic foods for the full 28-day. Participants, in addition to consuming supplements, completed a daily training and GI symptom diary.  

The study concluded that the prevalence of moderate GI symptoms was lower during the third and fourth weeks of supplementation, during the marathon, and URTI symptoms severity was lower in a probiotic group compared to the placebo group. They also found an direct correlation between exercise-induced GI permeability and symptoms. GI permeability was reported as the lowest symptom. In conclusion, recognize that GI permeability and damage may have clinical relevance, but there is no clear relationship between GI symptoms seen in the study.

**Probiotic Intervention**

This probiotic intervention contains the strains \textit{P. acidilatici}, \textit{CECT 7483}, \textit{L. Plantarum CECT 7484}, \textit{L. Plantarum CECT7485}, which were isolated from healthy children at the Universidad
Autonoma de Barlecolona. Prior to this study, this strain combination has previously been shown to improve symptoms in patients with IBS. These three strains were chosen because of their ability to survive gut passage and adhere to intestinal mucus in vitro. It also produces significant amounts of butyric, propionic, and acetic acid in a ratio similar to that found in the healthy gut. In addition, it has been shown to reduce inflammation and diarrhea in two different animal models.

Hypothesis

We hypothesized that 4 weeks of supplementation with the probiotic strains P. acidilatici, CECT 7483, L. Plantarum CECT 7484, L. Plantarum CECT7485 would be safe to use and would improve exercise-induced GI symptoms and associated biochemical molecules and result in alterations in the gut microbiome composition.

Therefore, the study is aimed at:

1. Investigating the effect of supplementation of probiotic supplementation in exercise-associated gut dysfunction through measurement of gut markers (i.e., Zonulin, IFABP, and LBP).

2. Determining if probiotics supplementation of results in microbiome changes to α and β diversity as well as to genera population.

3. Determining if probiotic supplementation alleviates exercise-induced gut discomfort and exhaustion as measured both by self-reported GI symptoms and the Borg Scale.
Materials and Methods

This study was a randomized placebo-controlled, double-blinded crossover study. Inclusion criteria was as follows; healthy men and women between 18-50 years of age; who have run for more than two consecutive years; run at least 15 miles a week; run three times or more per week; have completed a run of 90 minutes or more at least once per month for the past six months; and experiences GI discomfort during or after running (a rating of at least 4 on a scale of 0-9). Exclusion criteria includes: being pregnant or breastfeeding; using NSAIDs, probiotics or supplements that might alter the gut microbiome within one month before enrollment; following a low FODMAP (fermentable oligo, di-, monosaccharide, and polyols) diet, have a history of drug or alcohol abuse in the past year; have had GI surgery in the past year or have a history of severe heart, liver, kidney, neurological, oncological, psychiatric, coeliac or inflammatory bowel disease, acute pancreatitis, or are immunosuppressed.

Participants were recruited via social media posts, flyers, and word of mouth. After screening for eligibility, subjects provided informed consent and enrolled in the study. Anthropometric data was collected, and a urine pregnancy test was performed on all female participants. All participants then participated in a VO$_2$ max test to determine running parameters to be used on the testing days. For the VO$_2$ max test, participants ran in a temperature-controlled room (approximately 27°C) and heart rate and respiratory variables was measured using a metabolic cart (Parvo Medic) and a POLAR heart rate monitor.

Subjects returned to the lab for 4 blood sampling visits and 4 treadmill run tests. During each treadmill test, participants ran at an intensity of 70% of VO$_2$max for a maximum time of 90 minutes, in a temperature-controlled room (27°C) or until exhaustion, according to the Borg
scale or onset of GI symptoms. One day prior to the treadmill test, subjects came to the lab for a fasted (overnight) blood collection and were provided with an energy bar to consume prior to the run (participants requested Cliff bars). Participants were provided supplies for stool sample collection, needed for microbiome analysis. Participants then received the probiotic supplement or placebo to ingest for the following four weeks. After the 4-week period, participants returned to the lab for another fasted blood draw and treadmill run test. This was followed by a 4-week washout period. After the washout period, the protocol was repeated. See Figure 1 for experimental design.

Figure 1: Experimental design

Participants were instructed to maintain their habitual diet and lifestyle during the study period. Participants recorded their food and beverage intake for three days prior to each visit and diet was analyzed using FoodProcessor. Participants also completed a daily Bristol stool chart and a daily post-run GI distress questionnaire. In addition, participants filled out weekly GSRS forms throughout testing. The Gastrointestinal Symptom Rating Scale (GSRS) form is a clinical rating scale for gastrointestinal symptoms for patients with IBS and peptic ulcer disease.63
On days 0 (Baseline Test period 1), 28 (End Test period 1), 57 (Baseline Test period 2), and 84 (End Test period 2), participants reported to the laboratory in the morning after an overnight fast and blood was collected by venipuncture in serum separator and plasma tubes. Plasma was also be collected after the treadmill run on day 28 and 84. Plasma and serum were harvested and stored at −80 °C for batch biochemical analysis of the gut parameters including LPS binding protein, zonulin, and IFABP both from blood collected after an overnight fast and after the treadmill run. Commercially available kits were used for the measurement of lipopolysaccharide binding protein (LBP, HycultBiotech: HK315-02), zonulin (Alpco: 30-ZONSHU) and intestinal-fatty acid binding protein (I-FABP (HycultBiotech: HK406-02).

**Fecal Collection and Sample Preparation for microbiome analysis**

Fecal samples were self-collected by participants during the 24 hours prior to each ab visit. Fecal samples were collected using the OmniGENE -Gut system (OM-200, DNAGENOTEK). Collected fecal samples were stable at room temperature until delivery to the laboratory at which time the samples were stored at −80 °C.

For microbiome analysis, genomic DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (catalog # 51804, Qiagen) according to the manufacturer’s instructions. DNA was extracted by mechanical perturbation using a bead-beater (BioSpec Mini-beadbeater 16) for 3 minutes. DNA quantity and quality was determined by absorbance ratio at 260/280 nm using a Nanodrop (Fisher Scientific). Library generation and 16s rRNA sequencing will be performed at the Heflin Center for Genomic Science (University of Alabama, Birmingham), according to Kumar et al.
Amplicon libraries was prepared by PCR amplification of the V4 region of the 16S rRNA gene. PCR products were sequenced using NextGen sequencing Illumina MiSeq platform.

For microbiome analysis, FASTQ conversion of the raw data files was performed following de-multiplexing using MiSeq reporter. The quality assessment of the FASTQ files was done using FASTQC. Using the FASTX toolkit quality filtering was performed. Quantitative Insight into Microbial Ecology (QIIME) was used for analysis as described in Kumar et al.64

Within the QIIME, a combination of tools was used for clustering reads into operational taxonomic units, taxa assignment, alignment and phylogenetic inference using PyNAST. This procedure allows us to quantitatively assess the microbiome population down to the genus, and frequently species level. Data was imported into Calypso 8.84 for further analysis and visualization

**Statistical analysis**

Statistical analyses were performed using Graphpad Prism 9.1.2 and JASP 0.16. Linear Mixed Models were used to determine statistical significance. Statistical significance was established at p=0.05.

For microbiome analysis, Analysis of Variance (ANOVA) was applied to determine differences between groups for α-diversity. Between sample variation (β-diversity) was quantified using Bray-Curtis dissimilarly measure. Kruskal-Wallis test was used to determine differences in relative abundance between groups.
Results

One study concluded that based on a two-sided 5% significance level with 80% probability that only 8 participants were necessary to show significance. This research study followed the same conditions, so the same sample size was justified. However, in light of that study, the sample was increased as twenty-five participants were enrolled in the study and 17 complemented the study. Participants that had dropped out did not have any data collected and were therefore not included in the analysis. Participants were enrolled in the study between August 2020 and March 2021. Participants at baseline had a mean age of 33.2 ±8.2 years. 47% of the participants were males and 53% female. Participants had a mean bodyweight of 70.7±12.2 kg and BMI was 24.1±1.8 kg/m². Resting heart rate was 68.5±13.1 bpm. Mean Systolic and Diastolic blood pressures were 129.5±11.6 mmHg and 79.9±12.0 mmHg respectively.

Table 1: Subject characteristics at screening

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>33.2±8.2</td>
</tr>
<tr>
<td>Male</td>
<td>47 % (8/17)</td>
</tr>
<tr>
<td>Female</td>
<td>53 % (9/17)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>70.7±12.2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.1±1.8</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>68.5±13.1</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>129.5±11.6</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>79.9±12.0</td>
</tr>
</tbody>
</table>

Baseline data of 17 participants. Values are mean ±SD. (HR, Heart Rate; SBP, systolic blood pressure; DBP, diastolic blood pressure)
**Gut parameters**

Levels of blood zonulin, LBP and iFABP was measured after an overnight fast to determine if the probiotic supplement altered gut-associated molecules. Zonulin levels were 33.33 ±12.74 ng/ml at baseline and 34.040±8.61 ng/ml after one month placebo treatment and 30.13±12.03 ng/ml (baseline) and 29.63±11.92 ng/ml after one month of probiotic treatment. The probiotic did not result in statistically significant changes in fasting zonulin levels for run time (p=0.84), treatment (p=0.15) or treatment*time interaction (p=0.99, Figure 2).

**Zonulin**

![Figure 2: Fasting Zonulin](image)

Figure 2. Fasted blood zonulin levels. Zonulin measured in blood after an overnight fast as baseline and after 4 weeks of placebo or probiotic supplementation (placebo n=16, probiotic n=14). Significance determined using Linear mixed models.
**IFABP**

Blood I-FABP was measured after an overnight fast at the baseline and after 4 weeks of supplementation. At baseline, the Placebo measurement was 348.04±183.12 ng/ml, End Placebo was 435.30±277.67 ng/ml, baseline probiotic was 439.10±380.15 ng/ml, and probiotic end was 360.30±196.63 ng/ml. No significance was detected for time (p=0.95) and treatment (p=0.98). However, a time*treatment interaction was trending to significance (p=0.08) with a reduction in I-FABP with the probiotic treatment.

**Figure 3: Fasting IFABP**

Figure 3: Fasted blood IFABP levels. IFABP measured in blood after an overnight fast as baseline and after 4 weeks of placebo or probiotic supplementation (placebo n=16, probiotic n=14). Significance determined using Linear mixed models.

**LBP**

LBP was measured in blood after an overnight fast. Placebo mean at baseline was 10.64±4.40 ng/ml. end mean at baseline was 10.98±3.55 ng/ml. Probiotic baseline 14.0±8.1 ng/ml, and
probiotic end: 12.56±5.89 ng/ml. No significance was detected for time (p=0.53). treatment (p=0.63) or treatment*time (p=0.66).

Figure 4: Fasting LBP

Figure 4. Fasted blood LBP levels. LBP measured in blood after an overnight fast as baseline and after 4 weeks of placebo or probiotic supplementation (placebo n=16, probiotic n=14). Significance determined using Linear mixed models.

Pre and post Running

Treadmill run tests were completed in accordance with the 28-day schedules (see Figure 1) for each participant. An initial blood draw to test gut markers was completed on each individual applicant 24 hours prior to their treadmill run. Then, immediately following the treadmill run another blood draw was completed on each individual.
**Zonulin Pre/Post Run**

Zonulin levels were measured via blood draw 24hr prior and immediately after the treadmill run.

No significant changes were induced for either the placebo or probiotic group by the treadmill run (Figure 5). Mixed linear model analysis demonstrated a significant change in time (p=0.009), but no changes for either treatment (p=0.57) or time*treatment interaction(p=0.29). No significant changes were detected with exercise within the placebo or probiotic groups.

![Figure 5: Zonulin pre/post](image)

Figure 5: Exercise-induced zonulin levels. Zonulin levels measured pre-run and post-run via blood draw (placebo n=16, probiotic n=14). Significance determined using Linear mixed models.

**IFABP Pre/Post run**

IFABP levels measured via blood draw 24hr prior and immediately after the treadmill run.

Mixed linear model analysis demonstrated a significant change in time (p=0.01) and a trend towards significance were determined for time*treatment effect (p=0.07). No significance found with treatment (p=0.66). Within group analysis also showed a significant increase in IFABP with
the probiotics treatment (p=0.0144), not seen for the placebo group (p=0.79, Figure 6), suggesting that the probiotic treatment selectively increases IFABP with exercise.

Figure 6: IFABP pre/post

Figure 6. Pre run and post run IFABP levels. IFABP levels measured in blood before after treadmill runs (placebo n=16, probiotic n=14). Significance determined using Linear mixed models.

LBP Pre/Post run

LBP levels measured via blood draw 24hr prior and immediately after the treadmill run. Mixed linear model analysis demonstrated a significant change in time (p=<0.001). While no significance detected in treatment (p=0.68), and time*treatment (p=0.154). Within group analysis also showed a significant increase in LBP with both the probiotics treatment (p=0.0015) and for the placebo group (p=0.0002) seen in Figure 7. Exercise results in a significant increase in LBP independent of placebo or probiotic treatment.
Figure 7. Pre run and post run LBP levels. LBP levels measured in blood before after treadmill runs (placebo n=16, probiotic n=14). Significance determined using Linear mixed models.

**Microbiome**

Fecal samples were self-collected in the 24 hours prior to each lab visit. DNA was extracted and the V4 region of 16S ribosomal DNA sequenced for microbiome analysis. The majority of the gut microbiota in the study populations belonged to the phyla Firmicutes (61.5±15.04%) and Bacteroidetes (34.1±16%), with Actinobacteria (1.9±2.6%), Cyanobacteria (0.2±0.4%), Proteobacteria (1.6±0.9%), Tenericutes (0.1±0.15%) and Verrucomicrobia (0.6±0.128%) to a lesser amount. The major genera in the subject population were *Prevotella* (12.25±3.4%), *Bacteroides* (15.42±0.78%), *Blautia* (13.2±1.6%) and *Faecalibacterium* (6.9±1.1%). The bacterial composition is shown at genus level (20 most abundant genera) in figure 5 for the placebo group at baseline (Group 1) and endpoint (Group 2) and probiotic group at baseline (Group 3) and endpoint (Group 4) of the intervention.
Figure 8. Relative distribution at genus level of fecal bacteria collected from healthy male and female runners at the baseline (Group 1) and endpoint (Group 2) of placebo treatment and at the baseline (Group 3) and endpoint (Group 4) of the probiotic treatment. The 10 most abundant genera are listed.
α Diversity

α-Diversity was determined for samples at each time point. No significant changes were detected for Shannon (Figure 9A, p=0.83) or Simpson’s index (Figure 9B, p=0.74) between placebo and probiotic at any timepoint.

Figure 9A: Alpha Diversity Shannon Index

Figure 9B: α-Diversity Simpson’s Index

Figures 9. α-Diversity was measured in fecal samples collected at baseline and after 4 weeks of intervention using (A) Shannon diversity index and (B) Simpson’s diversity index. No significance was detected by ANOVA.
β Diversity

To compare diversity between samples, β-diversity were determined using the following metrics: Bray-Curtis dissimilarity (BC), unweighted Unifrac (UU) and weighted Unifrac (WU). No significant differences in diversity were observed (BC, p=1; UU, p=1; WU, p=0.911, data not shown). Data are represented on a principal component analysis plot (PCoA) showed no clustering with treatment (Figure 10).

Figure 10: PCoA Bray-Curtis genus

![PCoA Bray-Curtis genus](image)

Figure 10. Predicted principal components analysis (PCoA) plot of Bray–Curtis dissimilarity at genus level demonstrating β-diversity of the fecal microbiome across experimental time points. No clustering was observed for any groups.

Statistically significant differences in relative abundance were detected for individual genera. The following genera from the phyla firmicutes demonstrated changes:

*Hydrogenoanerobacterium* (p=0.00429), *Lachnospiraceae UCG-009* (p= 0.046), and *hydrogenoanaerobacterium_uncultered bacterium* (p=0.004), *Pediococcus* (p=0.026), and
*pediococcus pentosaceus* (p=0.006). Further, from the Bacteroidetes phyla, *Prevotella* also showed significance altered (p=0.0435).

Table 2: Differences in relative abundance

<table>
<thead>
<tr>
<th>OTU</th>
<th>P-value</th>
<th>Placebo</th>
<th>Probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time 0</td>
<td>Time 1</td>
<td>Time 0</td>
</tr>
<tr>
<td>Firmicutes; _Clostridia; Clostridiales; Ruminococcaceae; Hydrogenoanaerobacterium</td>
<td>0.00429</td>
<td>0.00016</td>
<td>0.00016</td>
</tr>
<tr>
<td>Firmicutes; _Bacilli; Lactobacillales; Lactoballaceae; Pediococcus</td>
<td>0.02673</td>
<td>0.00013</td>
<td>0.00013</td>
</tr>
<tr>
<td>Bacteroidetes; _Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella</td>
<td>0.04357</td>
<td>0.00016</td>
<td>0.00016</td>
</tr>
<tr>
<td>Firmicutes; _Clostridia; Clostridiales; Lachnospiraceae; Lachnospiraceae UCG-009</td>
<td>0.04687</td>
<td>0.00027</td>
<td>0.00027</td>
</tr>
<tr>
<td>Firmicutes; _Clostridia; Clostridiales; Ruminococcaceae; Hydrogenoanaerobacterium; uncultured bacterium</td>
<td>0.00429</td>
<td>0.00016</td>
<td>0.00016</td>
</tr>
<tr>
<td>Firmicutes; _Bacilli; Lactobacillales; Lactoballaceae; Pediococcus; Pediococcus pentosaceus</td>
<td>0.00626</td>
<td>0.00013</td>
<td>0.00013</td>
</tr>
</tbody>
</table>

Table 2. Data is relative abundance of individual microbes given as mean at Time 0 (baseline) and Time 1 (after 1 month of supplementation) of placebo or probiotic treatment. Significant differences are determined by the Kruskal-Wallis test.

**GI Discomfort**

Gastrointestinal discomfort during and after the treadmill run was determined using a questionnaire where symptoms were rated on a scale of 0-9 for both upper and lower GI symptoms. Symptoms for upper GI discomfort included reflux, belching, bloating, stomach pain/cramps, vomiting, and nausea. While lower GI symptoms included lower abdominal cramping, side ache/stich, flatulence, urge to defecate, and diarrhea. The participants reported symptoms every 10 minutes during their run and then completed a questionnaire at the end of the
run. Further, participants were also asked to assess their perceived levels of exertion via the Borg Scale, which was measured from 6-20 at each of the 10 minute intervals while running. All values of discomfort and Borg Scale were used to create figures 11-15 below. Table 3 summarized the findings via an area under the curve (AUC) analysis. Upper GI discomfort during the treadmill runs within the placebo period noticed a 27.19% reduction in area, whereas, in the probiotic period there was a 2.68% growth. While the percent change to area does not provide statistical significance, the variance amongst direction suggested that the probiotic may have worsened symptom experience within the participants. Lower GI Discomfort during the treadmill runs maintained trends of reduction for both placebo and probiotic, which were 5.53% and 29.77% respectively. Finally, Borg Scale calculations showed perceived exertion during the placebo period grew by 1.4%, while the probiotic lessened perceived exertion by 3.53%.

**Table 3: GI Discomfort and Borg Scale Values**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Placebo</th>
<th>Probiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End</td>
</tr>
<tr>
<td>Upper GI AUC</td>
<td>81.88±32.4</td>
<td>59.62±59.6</td>
</tr>
<tr>
<td>Lower GI AUC</td>
<td>112.2±40.0</td>
<td>106.0±44.4</td>
</tr>
<tr>
<td>Borg Scale AUC</td>
<td>1001±65.3</td>
<td>1015±58.3</td>
</tr>
</tbody>
</table>

Table 3. The calculated values are from Area Under the Curve Analysis utilizing Prism GraphPad. The measures in each category were the mean values associated with each category of symptom on the basis of the self-reported questionnaire from participants (placebo n=16; probiotic n=17). Run and Post run were measured across the individual treadmill runs. Baseline and End measures were tabulated at the end of each period of the study: the 4-week period of placebo or probiotic.

**Upper GI**

Figure 11 represented Upper GI Discomfort during the runs for each participant, which is averaged within each collection interval of 10 minutes. Across the 90 minutes, all 4 groups
seemingly overlap with very little variance in trend. Figure 12 illustrated the Post Run measures associated with Upper GI Discomfort. There were no major variances noted amongst the four groups.

Figure 11: Upper GI Discomfort During Run

Figure 11. Upper GI discomfort measures calculated via means across the 90-minute run time at 10min intervals. Participants self-reported scores (0-9) every 10 minutes during the treadmill run.

Figure 12: Upper GI Discomfort Post Run

Figure 12. Upper GI discomfort measures calculated via means provided through participants self-reported scores (0-9) after each treadmill run.
**Lower GI**

Figure 13 represented Lower GI Discomfort during the runs for each participant, which is averaged within each collection interval of 10 minutes. Across the 90 minutes, all 4 groups seemingly overlap with very little variance in trend. Figure 14 illustrated the Post Run measures associated with Lower GI Discomfort. There were no major variances noted amongst the four groups.

![Figure 13: Lower GI Discomfort During Run](image)

Figure 13. Lower GI discomfort measures calculated via means across the 90-minute run time at 10min intervals. Participants self-reported scores (0-9) every 10 minutes during the treadmill run.

![Figure 14: Lower GI Discomfort Post Run](image)

Figure 14. Lower GI discomfort measures calculated via means provided through participants self-reported scores (0-9) after each treadmill run.
Borg Scale

Figure 15 represented perceived exertion via the Borg Scale during the runs for each participant, which is averaged within each collection interval of 10 minutes. Across the 90 minutes, all 4 groups seemingly overlap with very little variance in trend. The Borg Scale exertion trend increased independent of placebo or probiotic across the 90-minute run.

Figure 15: Borg Scale

![Borg Scale Graph]

Figure 15. Borg Scale discomfort measures calculated via means across the 90-minute run time at 10min intervals. Participants self-reported scores (0-9) every 10 minutes during the treadmill run.

Discussion

Across each 4-week period (placebo and probiotic), multiple pieces of data were collected and analyzed. As can be seen in table 1, the participant demographic characteristics at the point of initial screening is highlighted. Namely, there is a relatively even split amongst age, gender, BMIs, and Cardiovascular health statuses. Thus, as a double-blinded placebo-controlled crossover study, the need to maintain demographic randomness within the participants was met. This gives both credibility and validity to the statistical findings within this study. Further, while
limited in participant number, the degree of variation within the participant pool reduces inherent bias as well as attempt to elucidate the efficacy of the probiotic strain regarding intestinal epithelial permeability and the alteration of the gut microbiome in a more generalizable, universal sense.

The first study aim was investigating the effect of probiotic use on exercise-associated gut dysfunction. This study corroborates many of the findings of previous research. Just as with each of those studies, markers for gut permeability (e.g., Zonulin, IFABP, and LBP) all were elevated in response to exercise in this study as well. This finding in corroboration with previous findings show a clear causal link between exercise and gut permeability. Further, this study corroborates the findings that probiotic use did not have a significant effect on the inflammatory response in that Zonulin, IFABP, and LBP are markers that were utilized to show an increase in intestinal inflammation. Namely, there was no measured significance outside of the within group analysis that indicated any therapeutic effect of the probiotic. As was seen in this study, data indicated no statistical significance for measures for Zonulin, IFABP, and LBP. However, complicating the established literature, the data offered points of significance. The within group analysis showed significance for IFABP and LBP. The within group analysis highlights internal significance within each respect group (i.e., placebo and probiotic) in the change associated for IFABP and LBP, but the significance cannot be utilized in comparisons between groups (i.e., placebo and probiotic in this study). The calculated significances of withing group analysis might be considered a false positive association otherwise. Further, the time measures for Zonulin, IFABP, and LBP all have significance. However, when compared with treatment, there is no measure of significance detected. This finding indicated that both pre and post run measures on their own have validity, but there is no significance with the utilization of this probiotic strain. Given that the within
groups significance was an important finding, the ultimate conclusion regarding a lack of significance within the probiotic strains use is in accordance with the established literature. Although one study indicates there was some GI inflammatory response as a decrease Zonulin specifically in some of the participants, they concluded no significance. Therefore, the data corroborates previous findings that indicated that there is no effect with the utilization of this probiotic strain and exercise-associated gut dysfunction.

The second study aim was investigating how this probiotic strain would alter the gut microbiome. Due to the uniqueness of the strain utilized within the study, it was difficult to compare to the findings of others in the field. As such, this probiotic must exist in its own space. However, based on the evidence, this probiotic did not offer therapeutic benefit. The closest research looking at this particular strain did not analyze the microbiome, which means this research study stood out as a unique perspective into the strain’s effects on the microbiota. The data illustrated that the associated microbiome alpha and beta diversities were not altered in any appreciable or statistically significant way during the placebo period compared with the probiotic period. Further, Figure 10 offered a Bray-Curtis dissimilarity where no associated clustering is appreciated. Genus distributions (Figure 8 and Table 2) of the most populous genera were analyzed. The *Firmicutes* colonies of *Hydrogenoanerobacterium, Lachnospiraceae UCG-009,* and *hydrogenoanaerobacterium_uncultered bacterium, Pediococcus,* and *pediococcus pentosaceus* were significant. Further, *Bacteroidetes* also showed significance. This indicated that further research should be utilized involving this strain to fully analyze its alterations and potential effects on *Pediococcus* colonies in the gut microbiome. Although there was no measured significance in alpha and beta diversity as well as no clustering on the Bray-Curtis,
there was a statistically significant effect on *Pediococcus* genera abundance, so further analysis and study should be done to fully investigate that effect.

Finally, the third study aim investigated if probiotic use alleviated any exercise-induced gut discomfort. Previous studies looked at the effects of probiotic use and the reduction of GI symptoms and/or run time.\(^{59,60}\) One study found no difference in race times within the participants but did note some reduction in GI symptoms experienced by participants.\(^{60}\) On the other hand, another found an increase in run time to fatigue.\(^{59}\) In light of those studies, the data in this study indicated there was no significant alleviation of exercise-induced gut discomfort, which corroborates the findings of Shing et al. (2014). The findings do not, however, show a significant reduction in GI symptoms from participants, which goes counter to the findings of Pugh et al. (2018). The data from this study elucidated the lack of alleviation of gut discomfort symptoms regarding both the Upper and Lower GI discomfort measures and the adjusted Borg Scale exhaustion measures. Some trends were noted within the data calculations. Firstly, the probiotic seemingly worsened Upper GI Discomfort symptoms via AUC. Secondly, the probiotic improved Lower GI discomfort more substantially than within the placebo control. Finally, via the Borg Scale, the probiotic may have alleviated some of the perceived exertion. That said, though, no statistical significance was noted on any of these measures as only AUC was performed to illustrate the findings. Therefore, there was no appreciated therapeutic benefit and no statistical significance to the utilization of the probiotic within these endurance athletes, which met the third study aim.

All three study aims investigated if there were any significant changes associated with the utilization of this bacterial strain. There was no therapeutic associated significance (significance associated with a reduction in GI Dysfunction and Discomfort) with the utilization of the
bacterial strain regarding the first and third study aim, which corroborated previous findings.51, 52, 53, 54, 55, 56, 57, 58, 59, 60

Conclusions
In conclusion, there were no therapeutic reductions in exercise-associated gut dysfunction, microbiome changes to both Alpha and Beta diversity, gut discomfort measures, and exhaustion as measured with the Borg Scale. That said, however, there was one interesting finding that existed outside of previous literature, which is that there was significance associated with \textit{Pediococcus} colony populations and the utilization of the probiotic. The probiotic strain used increases the population of \textit{Pediococcus} with a degree of significance, which indicated a need for further study. While the study did not provide any positive significance regarding the use of the probiotic, it was successful in identifying future research on the alterations to the \textit{Pediococcus} population, which is an interesting finding.

Limitations
As a final closing thought, this study has a serious limitation regarding the participant size, which would be the goal of a future research project. Perhaps by expanding the participant number, some of the measures trending to significance might reach significance. That said, though as indicated above, the varied and random participant pool does seem to eliminate some of the inherent bias associated with smaller participant studies, which would make the participant size of the research study a moot point.
References


doi:10.1016/j.chom.2018.05.012


doi:10.1016/j.tem.2016.03.001


   doi:10.1136/gut.49.2.159

   doi:10.1152/physrev.00003.2008


   doi:10.1038/nrgastro.2014.66


   doi:10.3390/ijerph110504745


56. West NP, Pyne DB, Cripps A, Christophersen CT, Conlon MA, Fricker PA. Gut Balance, a synbiotic supplement, increases fecal *Lactobacillus paracasei* but has little effect on


Appendix A

IRB Approval:

Institutional Review Board
Division of Research and Innovation
Office of Research Compliance
University of Memphis
315 Admin Bldg
Memphis, TN 38152-3370

PI: Marie van der Merwe
Co-Investigator: Maxime Paquette, Chidambaram Ramanathan, Keith Martin, Jacquelyn Pence
Advisor and/or Co-PI: Richard Bloomer
Department: College of Health Sciences
Study Title: Safety and efficacy of a probiotic supplement
IRB ID: PRO-FY2020-304
Submission Type: Renewal
Level of Review: Full Board

IRB Meeting Date: Jun 2, 2021 1:15:00 PM CDT
Decision: Approved
Approval Date: May 27, 2021
Expiration Date: May 27, 2022

Research Notes:
Findings:

The IRB has reviewed the renewal request. The University of Memphis Institutional Review Board, FWA00006815, has reviewed your submission in accordance with all applicable statuses and regulations as well as ethical principles.

Approval of this project is given with the following obligations:

1. If this IRB approval has an expiration date, an approved renewal must be in effect to continue the project prior to that date. If approval is not obtained, the human subjects consent form(s) and recruiting material(s) are no longer valid and any research activities involving human subjects must stop.
2. When the project is finished a completion form must be completed and sent to the board.
3. No change may be made in the approved protocol without prior board approval, whether the approved protocol was reviewed at the Exempt, Expedited or Full Board level.
4. Exempt approval are considered to have no expiration date and no further review is necessary unless the protocol needs modification.
5. Human subjects training is required every 2 years and is to be kept current at citiprogram.org.

Thank you,
James P. Whelan, Ph.D.
Institutional Review Board Chair
The University of Memphis.

Note: Review outcomes will be communicated to the email address on file. This email should be considered an official communication from the UM IRB.