Proposal: A Cross-over Trial Evaluating the Impact of Probiotic Supplementation with Fiber on Eating Habits

by

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Abstract

The role of probiotics is becoming very significant in the growth and maintenance of healthy gut bacteria; however, there is a lack in conclusive scientific evidence linking specific strains to health benefits. This project is a research proposal for a study to evaluate the impact probiotic and prebiotic supplements can have on eating behaviors, specifically satiety and food cravings, via gut bacteria. The hypothesis is that the daily use of probiotic supplementation, with fiber, will positively impact eating habits by decreasing food cravings and increasing levels of satiety. It has additionally been speculated that the inclusion of probiotic supplementation with fiber may have a direct impact on weight loss and control in obese individuals. Analyzing varying nutrition interventions for weight management, such as probiotic supplementation, can make it possible to provide evidenced-based dietary recommendations that can potentially improve a variety of risk factors, such as obesity or eating patterns with low levels of fiber, that directly impact an individual's health status.

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Chapter 1: Introduction

Obesity has become a world-wide epidemic, rapidly increasing within diverse populations annually. According to the CDC in 2016, prevalence of obesity among adults was 30-35%. More than half the states in the US reported greater than 30% obesity rates, including Wisconsin (CDC, 2016). Interventions for long term weight management have largely been ineffective up until this point. Studies have started to investigate a new angle on weight control related to manipulation of the gut bacteria in the intestinal tract.

The gut microbiota encompasses a variety of microbial species varying from individual to individual. This complicates developing and sustaining a process that supports healthy gut bacteria due to multiple influencing factors that need to be taken into consideration. The main regulatory site for the human appetite encompasses the gut and the arcuate nucleus of the hypothalamus in the brain, which is referred to as the "gut-brain axis." Signals traveling between the central nervous system and gastrointestinal tract are communicated via the gut-brain axis. Thus, studies have begun to evaluate if there is an impact on eating behaviors via the link between the gut and the brain.

Gut bacteria may influence eating behaviors by way of the gut-brain axis (Sanchez et al., 2017). Eating behaviors such as food responsiveness, enjoyment of eating, and satiety awareness have been observed in relation to energy intake and weight gain over time (Sanchez et al, 2017). Creating a healthy gut microbiome has been suggested to support optimal health outcomes such as improved weight control and reduced risk for obesity related diseases. Probiotic supplements and prebiotics have been evaluated for effectiveness in growth of optimal gut bacteria.

Integration of probiotics has been supported by evidence from previous clinical trials; however there is a need for additional evidence on the role of gut microbiome composition in disease prevention. The current proposed randomized controlled trial will examine the effect of incorporating a probiotic and prebiotic supplement on daily eating habits, alteration in gut bacteria, weight loss, metabolic disease risk, and overall emotional health status. The potential impact the microbiome has on mood regulation and weight reduction interventions will be studied. Additionally, the proposed study will evaluate the process and the possible health outcome(s) of supporting a healthy gut microbiome.

The results of this study could potentially provide additional evidence on the impact probiotic and prebiotic supplements can have on appetite control and mood regulation via gut bacteria. This study will provide further support on the use of probiotic and fiber supplementation for weight control in overweight or obese populations. Thus, dietitians could use this information to provide guidance and recommendations for probiotic supplements to clients and patients, along with diet and physical activity.

Research question

Does the daily use of a probiotic and fiber supplement, for seven days, impact eating habits such as food cravings (intensity, frequency, self-control), fullness, and hunger levels?

Hypothesis

The daily use of probiotic supplementation with fiber will positively impact eating habits.

Sub-problems

1. Will daily probiotic supplementation with fiber alter the composition of the gut microbiota significantly in the overweight/obese population?
2. Will probiotic supplementation with fiber reduce the risk for developing obesity related diseases?
3. Is a pre-planned diet containing specific amounts of nutrients necessary for the supplementation to be effective?

Limitations

1. The availability of enough resources and funding to be able to carry out this study.
2. The study results will be based on data collected from self-reported questionnaires.
3. The study results will assume participants will follow the study protocol and consume the probiotics and prebiotics in a timely manner.

Delimitations

1. This study will include 39 females who are classified by BMI as overweight/obese.
2. This study does not examine type of diet or physical activity participants normally follow.
3. This study will utilize a convenience sampling method.
4. This study will not control for the current health status of individuals.
5. This study will utilize specific brands for supplementation.
6. This study will run for three weeks, which is a shorter time frame.

Assumptions

1. Participants will answer the questionnaires honestly and within the prompted time frame.
2. If effective, the intervention will result in improved outcomes for the overweight/obese population.
3. The intervention will be applicable to the general public for preventative practice.
4. Results of this study will be meaningful and valuable to the field of dietetics, specifically in the weight management area.

Definition of Terms

Central nervous system (CNS): the part of the nervous system that consists of the brain and spinal cord. Sensory impulses are transmitted in, motor impulses are passed out, and coordination of activities throughout the entire nervous systems occurs here.

Dysbiosis: a microbial imbalance or maladaptation on or inside the body.

Gastrointestinal tract (GI): the part of the digestive system that consists of the stomach and intestines.

Gut-brain axis (GBA): consists of bidirectional communication between the central and the enteric nervous system, linking emotional and cognitive centers of the brain with peripheral intestinal functions.

Gut microbiota, gut flora, gastrointestinal microbiota: the complex community consisting of microorganisms that live in the digestive tract of humans.

Metabolic syndrome: a syndrome marked by the presence of at least three components (high blood pressure, abdominal obesity, high triglyceride levels, low HDL levels, and high fasting levels of blood sugar). These components are associated with increased risk of cardiovascular disease and type 2 diabetes.

Microorganism: an organism (typically a bacterium) of microscopic or ultramicroscopic size.

Prebiotics: a non-digestible food ingredient that promotes the growth of beneficial microorganisms in the gut microbiota and/or improvements in microbiome functions.

Probiotics: live microorganisms, taken as dietary supplements or found in fermented foods, that are similar to beneficial microorganisms located in the human gut.

Symbiosis: an interaction between two different organisms living in close physical association, typically to the advantage of both.

Chapter 2: The Literature Review

Introduction

With obesity being a world-wide epidemic impacting approximately half of the states in the country there is a dire need for successful long term weight management interventions. New approaches have started evaluating the effectiveness of manipulation of gut bacteria in the microbiome. The gut microbiota is composed of the largest population of microbes in the human body (Nicolucci, Hume, Martinez, Mayengbam, Walter & Reimer, 2017). It contains thousands of diverse species of bacteria which help regulate functions of the body. Disturbances within the gut microbiome can negatively impact these daily functions and cause adverse effects.

Alterations in the gut microbiota configuration have been associated with increased gut permeability, disturbed gut barrier functions, and increased plasma lipopolysaccharide concentrations, which increases risk of obesity and metabolic syndrome (Rivière, Selak, Lantin, Leroy & Vuyst, 2016). The connections between gut microbiota composition and increased risk for behavioral disorders such as: regressive autism, depression, schizophrenia, and depression are also being investigated (Rivière et al., 2016). Imbalances have been observed in the gut microbiome and cognitive areas of the brain in the presence of a disease. Brain crosstalk helps sustain homeostasis of the gastrointestinal tract (Carabotti, Scirocco, Maselli & Severi, 2015).

The gut and the brain are linked in what is referred to as the "gut-brain axis" (GBA) (see Figure 1). The gut-brain axis involves communication between the central nervous system and the gastrointestinal tract, which links emotional cognitive centers with intestinal functions. The main regulatory site for the human appetite is located in the arcuate nucleus of the hypothalamus in the brain, identified as the appetite control center. Along these lines, past studies support a

connection between the gut microbiota and weight control (Sanchez, Darimont, Panahi, Drapeau, Marette, Taylor & Tremblay, 2017). Evidence has suggested a healthy gut microbiota may play an imperative part in prevention of the development of metabolic diseases such as high blood pressure, excess fat around the waist, or high levels of blood sugar (Hulston, Churnside & Venables, 2015). Further, recent studies demonstrate the gut microbiota plays a critical role in regulation of energy balance within the body and incidence of excessive body weight (Sanchez et al., 2017). Therefore, additional examination of the gut microbiome's species could be beneficial for gaining knowledge on creating a healthy gut environment as a potential weight control intervention.

Developing and sustaining healthy gut bacteria is a complex process with multiple factors such as fiber intake and medication use, to take into consideration. Nutritional supplements such as probiotics and prebiotics have been examined for effectiveness in supporting growth of optimal gut bacteria. Probiotic supplements contain live bacteria that can play a beneficial role in the gut health of an individual such as, improved nutrient absorption, enhanced cognitive function, mood regulation, optimal digestion, and a decrease in systemic inflammation (Sanchez et al., 2017). Eating prebiotics and non-digestible fiber with the probiotics may also promote positive health benefits because the fiber serves as food for the probiotics once in the large intestine (Hulston et al., 2015). Interventions that optimize gut health may have a positive impact on eating behaviors of individuals.

Studies have closely examined if gut bacteria profiles are associated with eating behaviors (Sanchez et al., 2017). Eating behaviors such as food responsiveness (eating in response to environmental cues), enjoyment of eating, satiety awareness, eating in absence of hunger, eating disinhibition, and cognitive restraint have been observed in relation to energy

intake and weight gain over time in past studies. Incorporating probiotic supplements to alter the gut microbiome for better control over the dimensions of eating behavior could lead to better weight control at the individual level, eventually impacting incidence of obesity at the population level. The purpose of this literature review is to critically analyze the evidence on the effect of probiotic supplementation on the composition of the microbiome and dimensions of eating behavior.

Background

The gut microbiota is responsible for a number of beneficial functions to the host via the gut-brain axis, such as protecting against pathogen, shaping the intestinal epithelium, collecting energy, and regulating host immunity (Thursby & Juge, 2017). Mechanisms involving neuro-immuno-endocrine mediators control communication within the gut-brain axis (Carabotti, Bellis, Maselli & Severi, 2015). If these mechanisms are disrupted by an unstable gut microbiome the risk for intestinal and extra-intestinal diseases increases significantly. One of the major challenges for healthcare professionals in the field of weight management is providing an effective long term weight reduction program. Since lean and obese individuals differ in their gut microbiota, recent research studies have started to examine manipulation of the microbiota to control eating behaviors and prevent weight gain (Sanchez et al., 2017).

During an average life span approximately 60 tons of food and environmental microorganisms travel through the gastrointestinal tract causing a threat to gut integrity (Thursby & Juge, 2017). Further evaluation of the specific nutrients with the ability to alter gut bacteria is necessary to determine components to include within a nutritional treatment plan that support weight stabilization and gut homeostasis. Dysbiosis of conventional microbiota, an imbalance in

gut bacteria, can cause abnormal gut function and disruptions within the central nervous system, such as an induction of intestinal inflammatory cells or alteration in cognition (see Figure 2). Identifying factors that alter composition of the microbiome (diet, antimicrobials, or probiotics) could help lessen the risk or progression in disease stage to restore symbiosis in the gut (Thursby & Juge, 2017).

Establishment of Gut Bacteria

The human gastrointestinal tract is a complex ecosystem composed of bacterial species and genes (Quigley, 2013). There are two prominent phyla (Firmicutes and Bacteroides) forming approximately 3/4 of the microbiome (Carabotti et al., 2015). The key to a healthy gut microbiome is sustaining a balance among the thousands of diverse species of bacteria. Colonization of the microbiota is believed to begin during birth by mode of delivery (vaginal birth vs. assisted delivery), maternal, and environmental bacterial exposures (Quigley, 2013). A newborn's microbiota is composed of minimal diversity in bacteria, phyla Proteobacteria and Actinobacteria are the dominating types (Quigley, 2013). The microbiome continues to be rapidly populated by gestational age, feedings (breast milk vs. formula), changes in diet, illness, and exposure to antibiotics (Quigley, 2013). Around the age of two and a half, the diversity in species, composition, and functional abilities of an infant microbiome resemble those of an adult (Thursby & Juge, 2017). The microbiome is at an equilibrium in terms of bacterial abundance and diversity in adulthood with minimal significant changes if health conditions or gut environment are stable (Sharon, Sampson, Geschwind & Mazmanian, 2016).

The Gut-Brain Axis

Neural and hormonal lines of communication combine to allow the brain to influence activities of immune cells, epithelial cells, enteric neurons, and smooth muscle cells (Carabotti et al., 2015). Additionally, these cells are influenced by the gut microbiota through reciprocal communication supporting the existence of microbiome gut-brain axis (GBA) (Carbotti et al., 2015). The gut microbiota directly impacts the GBA through interactions with intestinal cells, enteric nervous system (ENS), and central nervous system (CNS) via neuroendocrine and metabolic pathways (Carabotti et al., 2015). The brain facilitates the influence on microbiota composition through secretions of signaling molecules by neurons, immune cells, and enterocromaffin cells (Carabotti et al., 2015). Furthermore, the brain plays an impactful role in controlling gut functions, such as secretion of acid, motility, and mucosal immune response, all imperative for preservation of the mucus layer where bacterial growth occurs (Carabotti et al., 2015).

Interactions between systems equally influence appetite sensations and secretions involved in the digestion process. Signals from the gastrointestinal tract are sent through sensory nerves and circulation to the brain (Ahima & Antwi, 2009). Gastrointestinal fullness and satiety are mediated by neuron communication between the nucleus of the solitary tract (NTS) projection to the visceral sensory thalamus and the visceral sensory cortex (Ahima & Antwi, 2009). In response to the presence of hydrochloric acid, amino acids, or fatty acids the gastrointestinal tract secretes hormones, such as Cholecystokinin (CCK) a satiety factor and Ghrelin an appetite factor, to regulate eating behaviors appropriately.

CCK stimulates the gallbladder to contract and release stored bile from the liver into the intestine which aids in the digestion and absorption of nutrients which directly impacts the gut microbiota composition (Ahima & Antwi, 2009). Ghrelin is a peptide hormone produced and released in the gastrointestinal tract which controls appetite. Ghrelin can trigger an increase in food intake by activating the arcuate nucleus cells in the hypothalamus and impacting other hormones related to metabolism, such as leptin and insulin (Ahima & Antwi, 2009). Thus, neuronal and hormonal signals within the GBA assure appropriate maintenance of gastrointestinal homeostasis and digestion. However, if there is a disturbance present such as a decrease in conversion of disaccharides to short-chain fatty acids (SCFA) for energy within the system, metabolic processes within the GBA will not function properly to be able to control appetite or satiety.

Altered expression and turnover of neurotransmitters caused by gene disturbances, in both nervous systems, is linked to a lack of microbial colonization since neurotransmitter receptors on bacteria are essential for communication between CNS effectors and bacteria to occur (Carabotti et al., 2015). This absence of colonization also generates alterations to gut sensory-motor functions such as delayed gastric emptying and intestinal transit (Carabotti et al., 2015). Additionally, signals sent from the nervous system may impact weight by indicating satiety cues to the brain, in turn altering metabolic processes such as caloric extraction from food (Lawrence & Hyde, 2017). Evidence supports probiotic species are associated with protection of the intestinal barrier and restoration of tight-junction integrity (Carabotti et al., 2015). Optimal gut health may close the gap between keeping the microbiome healthy and disease prevention. Therefore, researchers have been fascinated with evaluating modifications in eating behaviors via manipulation of the gut microbiota.

The Gut-Brain Axis Structure

The microbiome gut-brain axis involves multiple lines of communication within a complex structure. The CNS communicates along afferent and efferent autonomic pathways with varying intestinal targets such as enteric nervous system (ENS), immunity, muscle layers and gut mucosa, modulating motility, permeability and secretion of mucus (Carabotti et al., 2015). The enteric microbiota participates in bidirectional lines of communication with intestinal targets to control gastrointestinal functions (Carabotti et al., 2015). In parallel, the hypothalamic pituitary adrenal axis (HPA) is activated by environmental factors such as stress which produces cortisol (Carabotti et al., 2015). The HPA is driven by an interaction between amygdala (AMG), hippocampus (HIPP), and hypothalamus (HYP) which makes up the limbic system (Carabotti et al., 2015). HYP secretes corticotropin-releasing factor (CRF) stimulating the pituitary gland to secrete adrenocorticotropic hormone (ACTH) and trigger the adrenal glands to release cortisol (Carabotti et al., 2015).

Immunologic, Biochemical, Neuroendocrine Mechanisms within the Gut-Brain Axis

Immunologic Mechanisms

The gut microbiome influences brain health through a variety of mechanisms which can produce a positive or negative outcome. The microbial cell wall is composed of structural constituents that persistently trigger the innate immune system to produce cytokines, in response at the intestinal mucosal surface a basal state of immune activation is created which eventually impacts the entire body (Galland, 2014). Excessive cytokine levels may be associated with disrupted sleep (Galland, 2014). Depletion of protective gut bacteria leads to excessive stimulation of the innate immune system via lipopolysaccharides in response to dysbiosis or

increased intestinal permeability may lead to increased inflammation in the CNS (Galland, 2014). An increase in intestinal permeability and inflammation may cause a disturbance in typical neuroendocrine regulation, increasing the risk for disorders linked to abnormal CNS function (Galland, 2014).

Biochemical Mechanisms

There is a potential for encephalotoxicity through numerous metabolites produced by intestinal bacteria (Galland, 2014). Microbial fermentation of carbohydrate produces D-lactate, prior studies support increased levels of D-lactate producing bacteria were observed in the stool of individuals with chronic fatigue syndrome and neurocognitive dysfunction (Galland, 2014). Probiotics may limit production of D-lactate acid in the gut, however species should be chosen cautiously since Lactobacillus are D-lactate producers (Galland, 2014).

Colonic bacterial fermentation of ingestible carbohydrate produces an abundance of SCFAs specifically acetate, propionate, and butyrate (Galland, 2014). These SCFAs have widespread regulatory effects in the body through their influence on two systems of molecular signaling, inhibiting histone deacetylation (HDAC) and activating G-protein coupled receptors (GPCRs) (Galland, 2014). A fundamental process in the epigenetic regulation of gene expression includes the acetylation and deacetylation of histone proteins around DNA coils (Galland, 2014). Imbalances in HDAC have been associated with cognitive dysfunction, whereas inhabitation of HDAC may produce beneficial effects on CNS disorders (Galland, 2014). Transmission of information within cells to regulate cell behavior and recognition of molecules in the extracellular milieu are performed by transmembrane proteins (GPCRs) (Galland, 2014). GPCRs symbolize the major pathway where cells convert external cues into

intracellular signals to respond with appropriate actions (Galland, 2014). Thus, if a disruption occurs during this process risk for neurodegenerative disorders may increase (Galland, 2014).

Neuroendocrine Mechanisms

Gut microbes are capable of synthesizing and responding to hormones and neurotransmitters (Galland, 2014). Lactobacillus and Bifidobacterium produce gamma-amino butyrate (GABA), Streptococcus and Enterococcus produce serotonin, and Bacillus species produce norepinephrine and dopamine (Galland, 2014). These species responses influence microbial growth, virulence, and the control of infections (Galland, 2014). Widespread changes in gut microbial composition may be triggered by specific responses on potential pathogens (Galland, 2014). The mechanisms previously mentioned may trigger gut bacteria to influence function or dysfunction in the CNS (Galland, 2014). The CNS and neuroendocrine activity may, in response, stimulate growth of bacterial species and influence composition of the gut microbiome (Galland, 2014).

Immunology

Throughout the later stages in life the microbiome composition continues to be altered by nutritional, chemical, and immunological gradients along the gut (Thursby & Juge, 2017). The gastrointestinal tract limits exposure of the host's immune system to the microbiota by an intestinal barrier to maintain homeostasis and protect from damage (Thursby & Juge, 2017). Bacterial species located at the mucosal surface and within the mucus layer participate in interactions with host immunity (Quigley, 2013). The barrier consists of multiple unified mechanisms such as, physical (epithelial and mucus layers), biochemical (antimicrobial proteins and enzymes), and immunological factors (IgA and epithelial-associated immune cells) (Thursby

& Juge, 2017). Intestinal bacteria produce a variety of substances, such as SCFA, peroxides to highly specific bacteriocins, or proteases, which may inhibit potentially pathogenic bacteria or denature bacterial toxins (Quigley, 2013).

Increasing amounts of evidence suggest the gut microbiome has a strong impact on overall health status of an individual (Singh et al., 2017). Controlling host immunity is one way the microbiota can influence host health. The gut microbiome aids in immune cell recruitment and differentiation to regulate host immunity and immunologic diseases (Singh et al., 2017). Additionally, gut bacteria play an imperative role in immunomodulation, upregulation of cytoprotective genes, prevention and regulation of apoptosis, and maintenance of barrier function which all promote homeostasis of functions throughout the body (Quigley, 2013).

Metabolism

The bacterial species residing in the lumen participate in metabolic interactions with food or products of digestion which may impact host metabolic health (Quigley, 2013). Bacterial disaccharides' ability to salvage unabsorbed dietary sugars and convert them into SCFAs for energy within the colonic mucosa to promote growth of intestinal epithelial cells has been previously studied (Quigley, 2013). Additionally, intestinal dysbiosis has been associated with metabolic disorders such as obesity and diabetes (Singh et al., 2017). The production of neurotransmitters and neuromodulators from certain commensal organisms may modify gut functions, such as motility or sensation (Quigley, 2013). Thus, further evaluation of the microbiota's influence on the development and function of the CNS continues to progress.

Dietary Impact

Diet has been suggested to have a significant impact on gut bacteria composition and function of the microbiome (Galland, 2014). A strict change in diet modifies microbial composition within 24 hours of initiation and once diet is discontinued a return to baseline occurs within 48 hours (Singh, Chang, Yan, Lee, Ucmak, Wong, Abrouk, Farahnik, Nakamura, Zhu, Bhutani & Liao, 2017). With the ability to quickly identify and quantify gut bacteria species prior studies have produced supporting evidence of the impact diet has on host microbial composition (Singh et al., 2017). Through sequence analysis of microbial ribosomal RNA-encoding genes (16S ribosomal DNA) data revealed the adult microbiome is composed of five bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomicrobia organisms (Galland, 2014).

Prior studies have supported the ability of macronutrients (protein, fats, and carbohydrates) to produce shifts within the microbiome (Singh et al., 2017). Shifts in the microbiome with secondary effects on host immunologic and metabolic markers are triggered by proteins, carbohydrates, lipids, polyphenols, and probiotics (Singh et al., 2017). Diets containing large amounts of animal protein and fat produce an abundance of Bacteroides species and vegetarian diets produce Prevotella species (Galland, 2014). Protein consumption (animal or plant-based) was found to be positively correlated with diversity in microbial bacteria. Specifically, whey and pea protein increased Bifidobacterium and Lactobacillus. An increase in intestinal SCFAs which are anti-inflammatory and imperative for preservation of the mucosal barrier was also observed (Singh et al., 2017).

Numerous current diets, such as Mediterranean, vegan, Western, and gluten-free, have been assessed for their ability to modify bacterial composition in the gastrointestinal tract (Singh et al., 2017). After following a gluten-free diet for 30 days data supported populations of "healthy bacteria" Lactobacillus and Bifidobacterium decreased while populations of "unhealthy bacteria" E. coli and Enterobacteriaceae increased (Singh et al., 2017). Diets including fermentable plant-based foods, such as vegetarian or vegan revealed significantly lower counts of Bacteroides and Bifidobacterium species (Singh et al., 2017). The Mediterranean diet discovered increased levels of fecal SCFAs, Prevotella, and Firmicutes bacteria (Singh et al., 2017). Finally, consumption of the Western diet (higher in animal protein and fat, lower in fiber) caused a decrease in total bacteria, specifically Bifidobacterium and Eubacterium species (Singh et al., 2017). The impact of diet on the microbiome continues to closely investigate the metabolic effects related to obesity, diabetes, and cardiovascular disease (Galland, 2014).

Probiotics/Fermented Foods Impact

Probiotics are categorized as live microorganisms that contain beneficial properties to human health when consumed in sufficient quantities (Wu, Zhang, Yin & Ruan, 2017). Prebiotics are defined as a non-digestible food ingredient that promotes the growth of beneficial microorganisms in the gut microbiota and/or improvements in microbiome functions. Probiotics and prebiotics enhance the body's ability to absorb essential nutrients and trace minerals from foods eaten in turn supporting stabilization of an optimal health status. Fermented foods consisting of lactic acid bacteria, such as yogurt or cultured dairy products, may beneficially regulate intestinal health through their ability to trigger production of anti-inflammatory cytokines and impact pre-existing microbes in the gut (Singh et al., 2017). Based on these properties, foods enhanced for these regulatory microorganisms are referred to as probiotics.

Past evidence supports consumption of fermented foods increased the total population of bacteria in the microbiome, specifically Bifidobacterium and/or Lactobacillus species (Singh et al., 2017). Due limited scientific evidence that demonstrates which dietary intervention is best for stabilizing a healthy microbiome, additional evaluation of aspects of the diet microbiome relationship is necessary.

Microbiome Alteration and Health Outcomes

The species and diversity of gut bacteria varies widely between individuals and does not remain constant throughout life in direct response to individual's dietary intake (Lawrence & Hyde, 2017). Alterations to diet have been proven to quickly manipulate gut bacteria within a matter of days (Lawrence & Hyde, 2017). Thus, assessing eating patterns becomes an essential part of gut bacteria manipulation. Interventions focusing on caloric reduction, inclusion of probiotic and/or prebiotic supplementation, and weight loss programs have produced beneficial health outcomes post study. For example, dietary modifications were associated with a greater diversity in composition of gut bacteria (Lawrence & Hyde, 2017). These modifications included restriction of processed carbohydrate foods, sugar, and artificial sweeteners. To further support a favorable change in gut bacteria composition, dietary modifications also included an increase in consumption of vegetables and fermented foods (Lawrence & Hyde, 2017).

Microbial metabolites, such as fecal bile acids (FBAs), are a possible mechanism which affects host physiology through alterations in gut microbiota (Nicolucci et al., 2017). Increases in primary FBAs have been associated with negative outcomes including diarrhea and a decrease in Bifidobacterium. Despite the lack of strong evidence to support what constitutes a healthy microbiota, certain species such as Bifidobacterium spp. and Lactobacillus have been

recognized as beneficial. There was a positive correlation between a reduced amount of Bifidobacterium and individuals who are obese (Nicolucci et al., 2017).

While current eating habits paired with sedentary lifestyles contribute to an increase in the obesity rate, researchers are discovering other important variables in prior studies to assess for potential associations. Variables include host genetics, severity of caloric depletion in eating patterns, degree of weight change, and duration of the study (Clarke, Murphy, Nilaweera, Ross, Shanahan, O'Toole & Cotter, 2012). Diet related variances between obese and lean individuals in the composition of the gut microbiota could be the most important factor to evaluate (Clarke et al., 2017). In adults, the gut microbiome is composed of bacteria belonging to two phyla species, the gram-negative Bacteroidetes and the gram-positive Firmicutes (notably Lactobacillus); and other bacterial species belong to the Actinobacteria group (Belizario & Napolitano, 2015).

Prior studies evaluated the link between obesity and the composition and functionality of microorganisms in the gut (Clarke et al., 2012). This was completed through 16S rRNA sequencing of DNA extracted from fecal samples of obese and lean individuals (Clarke et al., 2012). Results revealed dominance in Bacteroidetes and Firmicutes species in the microbiota (92.6%), but lower amounts of Bacteroidetes in the obese individuals compared to lean along with higher amounts of Firmicutes in the obese individuals compared to lean (Clarke et al., 2012). Additionally, obese individuals were found to have elevated quantities of Actinobacteria and less diversity in the microbial population of the gut compared to lean individuals (Clarke et al., 2012). The existence of inconsistencies between prior evidence on the link among gut microbiota and obesity has hindered the ability to define potential treatments that target specific components of the gut microbiota of obese individuals for weight reduction (Clarke et al., 2012). Therefore, further studies are needed in order to confirm whether particular quantities of

bacterial species are needed to develop and maintain a healthy gut microbial community to support normal body functions.

Manipulation of the gut microbiota composition through probiotic supplementation to favorably impact the gut-brain axis may be a means to control eating behavior traits and support substantial weight loss. The incorporation of probiotic supplementation could potentially have beneficial effects on appetite control and related eating behaviors (Sanchez et al., 2017). Supplementation with prebiotics maintains homeostasis in the gut microbiome, stabilization of fecal bile acids (FBA), and hopefully creates a healthy microbiota (Nicolucci et al., 2017). Nicolucci et al. (2017) implemented a 16-week period which revealed Actinobacteria significantly increased from baseline between the prebiotic group (13.5% ± 8.6) compared to the control (9.5% ± 6.4) ($p = 0.008$). Additionally, the change in Bifidobacterium from baseline was significantly different between the prebiotic group (9.843% ± 6.242) compared to the control (6.655% ± 6.168) ($p = 0.012$) (Nicolucci et al., 2017). Thus, Nicolucci et al. (2017) concluded daily use of prebiotics for a 16-week period is able to modify microbiota of overweight children. Therefore, continued evaluation of the effectiveness in the inclusion of specific probiotic and/or prebiotic genera that alter the microbiome and reduce disease symptoms can help determine nutritional practices to implement within the field.

Weight Reduction/Control

The role the gut microbiota plays in the regulation of energy balance and incidence of extra body weight has been analyzed to be able to identify potential associations (Sanchez et al., 2017). Results support women under diet supervision with a probiotic supplement experienced an increased weight loss of (mean = -5.2 kg) compared to the control (mean = -2.5 kg) (Sanchez

et al., 2017). Therefore, it has been proposed that prebiotics and probiotics can be incorporated with dietary intake to manipulate gut microbiota for prevention of weight gain (Sanchez et al., 2017).

Studies have recently begun exploring the influence of probiotic supplementation on weight. In particular, Lactobacillus species have been associated with promoting normal weight due to its ability to survive throughout the gastrointestinal tract (Madjd et al., 2016). Chang et al. (2011) suggested probiotic species (Streptococcus thermophilus, Lactobacillus acidophilus, Bifidobacterium infantis, Bifidobacterium breve, and Enterococcus faecalis) are beneficial for weight control, improved energy metabolism, obesity treatment, and reduced cholesterol levels. Use of prebiotics was associated with a significant improvement in satiety, which is closely connected to energy intake (Nicolucci et al., 2017). Additionally, consumption of a diet including probiotic and prebiotic items may improve weight management and reduce risk of obesity related diseases through creating healthy gut bacteria (Nicolucci et al., 2017).

In addition to the use of probiotics and prebiotics, another technique to alter the gut is fecal microbiota transplantation (FMT) (Marotz & Zarrinpar, 2016). The process of a fecal transplant entails obtaining a fecal matter sample from a healthy individual to transfer to a diseased individual in hopes of alleviating the illness (Marotz & Zarrinpar, 2016). Data suggests obesity is connected with extensive changes in the composition and metabolic function of the gut microbiota (Vrieze et al., 2012). When the gut microbiota was examined in a study assessing obese mice, it revealed a decrease in microbial diversity directly impacting digestion of carbohydrates, weight, and insulin sensitivity (Vrieze et al., 2012). Transplantation of a lean donor's fecal sample increased gut microbiota diversity, in turn improving the host's energy

metabolism and insulin sensitivity (Vrieze et al., 2012). Thus, a fecal transfer from a healthy person to an overweight person may support weight loss.

Clinical Relevance

The effectiveness of probiotic supplementation to treat metabolic, inflammatory, gastrointestinal, allergic, and respiratory conditions has been examined in past clinical trials. Studies specifically looked at the efficacy of supplementation in reduction/elimination of symptoms typically associated with these disorders.

Probiotic Lactobacillus

Probiotic strains, Lactobacillus and Bifidobacterium, have been studied for health promotion (Shirouchi, Nagao, Umegatani, Shiraishi, Morita, Kai, Yanagita, Ogawa, Kadooka, Sato, 2015). Lactobacillus contains numerous functional properties such as antioxidant properties, antimicrobial activity against pathogens, improvement in metabolism and cholesterol-lowering effects (Shrarfedtinov et al., 2011). Lactobacillus may have an impact on weight regulation due to its involvement in the fermentation of sugars into acids (Lawrence & Hyde, 2017). Furthermore, many Lactobacillus strains have previously been shown to prompt transcriptional initiation of fatty acid ß-oxidation related genes found in the muscle and liver (Shirouchi et al., 2015). These past findings suggest the supplementation of probiotic strains, specifically Lactobacillus, may support optimal health outcomes.

Probiotic Bifidobacterium

Another frequently used probiotic is strains of Bifidobacterium species found in food items (dairy products) and supplements. Bifidobacteria has been shown to improve digestive

problems and disorders, acceleration of the gut transit time, reduce antibiotic-associated diarrhea, and reduce irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) symptoms (Rivière et al., 2016). Previous studies have shown a decreased number of Bifidobacterium and butyrate-producing bacterial species in the colon in patients with infectious diseases. Treatment has focused on stimulation of the colon bacteria to restore a disturbed gut homeostasis and prevent further instabilities (Rivière et al., 2016). The most common interventions include consumption of probiotics and/or prebiotics within the daily diet (Rivière et al., 2016). However, it is imperative to take into consideration the probiotic health benefits from the Bifidobacterium strains may be caused by a variety of bacterial interactions within the gut microbiota.

Clinical Application

Past evidence creates the potential for healthcare professionals to recommend the use of a probiotic and/or prebiotic sources for changes to eating behaviors and overall health promotion. Unfortunately, it remains unclear whether the beneficial probiotic effects are facilitated by altering the gut microbiome composition directly, due to inconsistent results between studies (Lawrence & Hyde, 2017). This supports the need to further examine microbial changes that occur in response to probiotics and/or prebiotics through specific evaluation of the link between eating patterns, bacterial species, and the microbiome.

Current Research on Probiotics/Prebiotics to Support Healthy Gut Bacteria

The human intestines are filled with trillions of different strains of gut bacteria. Sub-optimal gut bacteria may be associated with negative long term health outcomes (Lawrence & Hyde, 2017). The current trend of probiotic supplementation for a healthy gut microbiome has led to a variety of health benefits to be analyzed and assessed. Previous research which

examined the incorporation of probiotic bacteria in rodents suggest improvement in metabolism and anti-obesity methods (Shirouchi et al., 2015).

To support favorable bacterial colonies in the human gastrointestinal tract, probiotics have been paired with prebiotics to increase the amount of Bifidobacteria and butyrate-producing bacterial species present in the colon (Rivière et al., 2016). These specific probiotic species have been shown to play a role in a variety of functions within the body. Therefore, it could be beneficial to support growth of Bifidobacterium colonies to reduce risk of disorders in direct response to disruption in the gut microbiome.

Prebiotics have been identified as "non-digestible food ingredients, dietary fiber, that aid in the stimulation and growth of bacteria in the colon to improve host health" (Slavin, 2013). Recently, the definition was advanced to "a fermented ingredient that supports specific changes, both in the composition and activity of particular microorganisms in the gastrointestinal microbiome" (Slavin, 2013). The most common microorganism genera for prebiotics are Lactobacillus and Bifidobacteria due to the abundance in population within the gut (Slavin, 2013). Prebiotics have the ability to stimulate fermentation in the colon and trigger favorable growth or activity of gut bacteria, such as Bifidocateria and Lactobacilli (Slavin, 2013). Further benefits of prebiotics include decline in potentially pathogenic bacteria population such as clostridia, enhancement in SCFA production, and enhancement in gut barrier function and hot immunity (Slavin, 2013). In order to identify whether or not prebiotics enhance health outcomes additional investigation of prebiotics role in influencing gut bacteria is essential.

Dietary Modification and Probiotic Supplementation for Weight Loss

In 2017, the Department of Psychology at St. Mary's University in the United Kingdom performed a retrospective uncontrolled observational study to see whether a dietary intervention, intended to influence gut bacteria, had a positive effect on physical and emotional well-being in healthy adults (Lawrence & Hyde, 2017). The study included 21 healthy participants (20 females and one male) that had all undertaken group nutritional therapy for clinical practice, ages 27 to 64 years, 20 were Caucasian and one Asian, and all participants were seeking services of a nutritional therapist. Individuals were excluded from the study if they were not taking part in group nutritional therapy. Throughout the four-week study period, participants followed a dietary program called the Gut Makeover diet, which is the restriction of processed high grain carbohydrate foods, sugar, and artificial sweeteners while increasing the consumption of vegetables and fermented foods to manipulate gut bacteria. Symptoms related to the digestive tract, the mind, and emotions were assessed using the functional medicine medical symptoms questionnaire (MSQ). The MSQ was also used as a tracking tool to evaluate participant progress to dietary changes.

Participants lost a significant amount of weight by the end of the intervention (female mean = 65.3 kg, male = 95.1 kg, SD = 9.78 kg) compared to the pre-intervention (female mean = 68.5 kg, male = 99.2 kg, SD = 10.68 kg) ($p< 0.0001$). There was also a significant reduction following the dietary intervention on medical symptoms, such as dizziness, diarrhea, and binge eating based on the MSQ scores. Severity of medical symptoms reported after the intervention decreased more than 3 times (mean = 18.71, SD = 12.69) compared to pre-intervention severity (mean = 31.95, SD = 31.95) ($p<0.0001$) (Lawrence & Hyde, 2017). Significant improvements were reported in participants' emotions as well. The authors concluded the Gut Makeover diet

was associated with a positive impact on gastrointestinal (GI) symptoms, weight control, and mood (Lawrence & Hyde, 2017). Improvement in emotional well-being from dietary modifications was also consistent with past studies which implemented more direct methods to alter gut bacteria (Lawrence & Hyde, 2017). Unfortunately, overall energy intake was not recorded in order to determine if a caloric reduction was present, which if so, was likely the reason for the weight loss and improved well-being.

The length of this study, four-week intervention, is a strength because it was long enough to illustrate how dietary modifications can impact health outcomes, while not effecting the attrition rate. Another strength is the usage of a programmed dietary intervention that provided guidelines that were simple and easy to follow. A limitation of this study is the sample size only being 21 participants and mostly females, which makes it difficult to extend to the general population. Another limitation is not adjusting for confounding variables such as baseline eating pattern or physical activity routine that may have impacted the results. Because neither the researchers nor the participants were blinded and no control group was used, placebo-effect may have occurred. Since gut bacteria was not assessed, it is unclear if gut bacteria changed as a result of the diet program and if that lead to improved health outcomes. Therefore, further research utilizing biomarkers is necessary in order to determine the direct effect a specified diet can have on the gut microbiome.

In 2016, a randomized, double-blind, placebo-controlled trial to examine the effects of a weight-reducing program with probiotic supplementation on mood and eating behaviors of obese individuals was conducted (Sanchez et al., 2017). The study included 105 obese men and women ages 18 to 55 years, of stable body weight, with a BMI between 29 to 41 kg/m2, and without associated co-morbidities (Sanchez et al., 2017). The study was 24-weeks with a weight

loss phase and weight maintenance phase. Participants were randomly assigned to the treatment or control group. All participants received a personalized diet plan with a 500 kcal/day deficit for the weight loss phase and during the maintenance phase there was no energy restriction. For the duration of the study, the intervention group was administered two probiotic supplement capsules per day (Lactobacillus rhamnosus CGMCC1.3724 (LPR)) while the control group was administrated two placebo supplement capsules per day. Both groups were instructed to take one capsule 30 minutes before breakfast and one capsule 30 minutes before dinner.

Appetite sensations were measured with the self-reported Visual Analogue Scales. Dietary intake and physical activity were measured by three-day records. Body composition was assessed by dual-energy x-ray absorptiometry. Eating behaviors were measured with the Three-Factor Eating Questionnaire and food cravings were measured by the State-Trait Food Cravings Questionnaire. Lastly, mood components were measured through a variety of questionnaires: the Beck Depression Inventory (BDI), the Body Esteem Scale, the Binge Eating Scale, and the State-Trait Anxiety Inventory (Sanchez et al., 2017).

Results showed the LPR supplement increased weight loss in women from baseline (mean = -5.2 kg) compared to the control (mean = -2.5 kg) and the LPR group was associated with an increased fasting desire to eat (mean = 22.2) compared to the control (mean = 6.8) ($p = 0.03$) (Sanchez et al., 2017). Whereas, in men there was no effect of the LPR treatment on body weight. Satiety levels in the female LPR supplement group increased at lunch compared to the control ($p = 0.02$), while disinhibition ($p = 0.05$) and hunger ($p = 0.02$) scores in the female LPR group decreased more when compared to the control (Sanchez et al., 2017). Furthermore, the female LPR group displayed a higher decrease in food cravings (mean = -23.2) compared to the control (mean = -12.4) ($p = 0.05$), a significantly lower depression score in the female LPR

group (mean = -1.4) compared to the control (mean = 0.9) ($p = 0.05$), and an increase in the body esteem score in the LPR group (mean = 11.0) compared to the control (mean = 7.3) ($p = 0.06$) (Sanchez et al., 2017).

In the male LPR group, fullness in the fasting state was higher ($p = 0.02$) and cognitive restraint was improved ($p = 0.01$) compared to the control (Sanchez et al., 2017). The authors concluded LPR supplementation improved emotion-related behaviors, satiety, and amount consumed (Sanchez et al., 2017). The results of lower scores in BDI, hunger sensations, and disinhibition in the LPR-treated women strongly supported their conclusion. On the other hand, mood results relied heavily on self-reported subjective questionnaires which could impact the validity of the outcomes. Authors concluded a weight loss program with probiotic supplementation was associated with a positive influence on eating behaviors, appetite sensations, and mood.

The length of this study, 24 weeks, is a strength because this is an efficient time frame to be able to observe if a weight-reducing program with probiotic supplementation impacted mood and eating behaviors of obese individuals through questionnaire results. Another strength is the number of participants. A limitation of this study is the lack of biomarkers, such as ghrelin or leptin levels, and gut bacteria content, in order to strengthen reliability of results. Another limitation is the study did not determine whether probiotics or prebiotics had a greater influence on appetite sensations and eating behaviors. Past studies found food cravings as both an obstacle to weight loss and the cause for regain after weight loss. Therefore, the theory that decreased food cravings may help maintain weight loss over time is partly consistent with previous studies.

In 2016, a randomized, single-blind, controlled trial compared the effect of probiotic yogurt with low-fat yogurt consumption on body weight and cardio-metabolic risk factors during a weight reduction program (Madjd et al., 2016). The study included 89 overweight and obese women, ages 18 to 50 years, BMI 27 to 40 kg/m2, and who normally consumed low-fat yogurt in their diet (Madjd et al., 2016). In combination with a weight reduction program, women were randomly assigned to the probiotic yogurt group or low-fat yogurt group for a 12-week period. The diet program introduced a 500 to 1000 kcal energy deficit based on the individual's baseline estimated energy requirements. Physical activity levels gradually increased, throughout the study, to achieve 60 minutes of moderate activity five days per week. Anthropometric measurements and blood samples (fasting plasma glucose, glycated hemoglobin (HbA1c), total cholesterol, triglycerides, and 2-h postprandial glucose (2hppG)) were assessed (Madjd et al., 2016).

After the 12-week intervention, there were significant reductions in weight, BMI, and waist circumference in both groups compared to baseline ($p < 0.001$) (Madjd et al., 2016). However, none of the results were significantly different between the two groups at the end of the study: weight loss ($p = 0.248$), BMI ($p = 0.296$), and waist circumference ($p = 0.269$) (Madjd et al., 2016). Reductions were seen in each group in a variety of lipid level profiles after the 12-week period compared to baseline. Compared with the LF group, the PY group had significant improvements in total cholesterol ($p = 0.024$) and LDL cholesterol ($p = 0.018$) (Madjd et al., 2016). Furthermore, data supported a decline in fasting plasma glucose, 2hpp glucose, HbA1c, fasting serum, and HOMA-IR after the 12-week period in each group (Madjd et al., 2016). Significant reductions between the two groups compared to baseline were shown for 2hpp glucose ($p < 0.001$), serum insulin ($p = 0.002$), and HOMA-IR ($p = 0.002$) after the 12-week

intervention (Madjd et al., 2016). The authors concluded that there was no significant effect on weight loss from consumption of probiotic yogurt compared to low-fat; however, when paired with a weight-loss program, probiotic yogurt could have positive effects on lipid profiles and insulin sensitivity (Madjd et al., 2016). Further analysis is necessary before application in practice can occur.

The design of the study, a randomized outpatient clinical trial, is a strength because randomization increases the likelihood that treatment and control group are equivalent at baseline. This is one reason a randomized controlled trial design is also known as the "gold standard" for clinical trials. Another strength is recruiting a population that was interested in weight loss, since this strengthened compliance with following the meal plan for the entire study period. A limitation of this study is the sample population because it included solely women which made it hard to extend results to the general public. Another limitation is the length of the study, 12-weeks, which did not efficiently demonstrate any long term effects. The study's results of no significant difference in weight reduction were consistent with former reviews in the dietetics research field on the effect of probiotics on obesity. Future examination is essential in order to conclude stronger associations, or lack of, between probiotic yogurt and weight loss.

Prebiotic Supplementation for Disease Prevention

In 2014, the faculty of Kinesiology at the University of Calgary in Canada performed a single-center, double-blind, placebo-controlled trial of two separate cohorts designed to examine childhood obesity. The purpose of this study was to assess if gut microbiota alterations occurred from prebiotic supplementation in overweight and obese children (Nicolucci et al., 2017). The study included 38 children ages 7 to 12 years, BMI >85th percentile, but otherwise healthy

(Nicolucci et al., 2017). Participants were randomly assigned to either the prebiotic oligofructose-enriched insulin (OI group) or maltodextrin placebo for 16-weeks (Nicolucci et al., 2017). Fat mass and lean mass were measured by the dual-energy x-ray absorptiometry. Blood samples were collected to analyze: lipids, cytokines, lipopolysaccharide, and insulin. Fecal samples were collected to analyze the composition of the microbiota through DNA sequencing and bile acids were profiled with a high-performance liquid chromatography (Nicolucci et al., 2017).

There were significant differences in the primary fecal bile acids between the OI group and the placebo group at completion of the study. For cholic acid (CA) the prebiotic group had a mean of 2.290 ± 0.949 compared to the control mean of 3.374 ± 1.533 ($p = 0.043$). For chenodeoxycholic acid (CDCA) the prebiotic group had a mean of 1.246 ± 0.739 compared to the control mean of 2.539 ± 1.699 ($p = 0.008$). Actinobacteria significantly increased from baseline between the prebiotic group (13.5% ± 8.6) compared to the control (9.5% ± 6.4) ($p = 0.008$). The change in Bifidobacterium from baseline was significantly different between the prebiotic group (9.843% ± 6.242) compared to the control (6.655% ± 6.168) ($p = 0.012$) (Nicolucci et al., 2017).

After 16 weeks there was a 3.1% decrease in body weight and 2.4% decrease in body fat (Nicolucci et al., 2017). These changes were significantly and positively correlated with a change in Clostridium clostridioforme from baseline between the prebiotic group (1.998% ± 1.347) compared to the control (2.517% ± 1.140) ($p = 0.024$) (Nicolucci et al., 2017). There was a 3.8% decrease in trunk fat which was significantly and positively correlated with a change in Bacteroides vulgatus from baseline between the prebiotic group (0.459% ± 0.522) compared to the control (0.339% ± 0.456) ($p = 0.005$) (Nicolucci et al., 2017). Along with a change in

bacterium mpn-isolate from baseline between the prebiotic group (0.014% ± 0.017) compared to the control (0.012% ± 0.016) ($p = 0.016$) (Nicolucci et al., 2017). The prebiotic supplement group also saw a 15% decrease in interleukin 6 and a 19% decrease in serum triglycerides which were both significant compared to baseline (Nicolucci et al., 2017). The authors concluded OI supplements prompted gut bacterial shifts and improved obesity outcomes in children classified as overweight/obese (Nicolucci et al., 2017). Insufficient data exists on modification of the gut bacteria for weight control in obese children; therefore, additional research is needed to determine potential interventions for this population.

The length of the study, 16-weeks, is a strength because it provided multiple opportunities to collect measurements throughout the study. Another strength is the use of blood and fecal samples as biomarkers to strengthen the study results. A limitation of this study is the incorporation of otherwise healthy children; this limits the ability to extend results to the greater population. Another limitation is the irregular bowel movements seen within participants which made it hard to control time of day for fecal collections. The current study's results of a reduction in body fat percentage with the OI group were consistent with past studies that aimed at childhood weight loss with OI supplementation (Nicolucci et al., 2017). However, further research with a more diverse sample size is necessary in order to make results applicable in practice.

Probiotic and Prebiotic Supplementation for Treatment

In 2015, the Department of Nutrition at Iran University in Tehran, Iran performed a randomized, double-blind, placebo-controlled study to examine individuals with non-alcoholic fatty liver disease (NAFLD) and the impact probiotic and prebiotic supplementation could have

on adipokines and glycemic parameters (Behrouz, Jazayeri, Aryaeian, Zahedi & Hosseini, 2015). The study included 89 patients with NAFLD, 20 to 60 years old, BMI >/= 25 kg/m2 and </= 40 kg/m2 with no recent weight loss over the last three months or no past history of weight loss surgery. Individuals were excluded from this study if they were pregnant, in lactation stage, consumed omega-3 fatty acids or supplements in the past year, comorbidities that are not appropriate: chronic or acute disorders of the liver, cirrhosis, celiac disease, diabetes, hypertension, cardiovascular disease, kidney disease or lung disease, alcohol abuse, use of antibiotics over a week, contraceptive pills, corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs), and significant changes in the recommended diet and daily physical activity levels. Throughout the 12-week study period, participants were randomly dispersed into three intervention groups; probiotic capsule with prebiotic placebo, probiotic capsule with prebiotic powder or probiotic placebo capsule with prebiotic placebo. Participants were offered diet and physical activity recommendations since general treatment for NAFLD emphasizes lifestyle interventions. Adiponectin, leptin, serum insulin, fasting glucose, BMI, and body fast percentage were assessed to evaluate if significant metabolic changes occurred.

Participants in the probiotic and prebiotic intervention groups experienced a significant improvement in parameters compared to the control group at the end of the intervention. Leptin levels decreased significantly in the probiotic and prebiotic groups ($p < 0.001$), insulin amounts decreased significantly in the prebiotic group ($p <0.001$), and HOMA-IR decreased significantly in the probiotic and prebiotic groups ($p <0.01$, $p <0.001$). There was also a significant increase in the probiotic and prebiotic groups in insulin sensitivity measured by QUICKI ($p <0.001$). There were no significant changes observed in adiponectin or fasting blood sugar levels following either intervention according to the ANCOVA completed ($p = 0.296$ and $p = 0.837$)

when compared to the control group (Behrouz et al., 2015). There were significant reductions in weight, body fat percentage, body mass index, physical activity, and energy intake at the beginning and end of the intervention within each group (Behrouz et al., 2015). The authors concluded there were beneficial effects on insulin metabolism and serum leptin with the administration of probiotic and prebiotic supplementation (Behrouz et al., 2015). Unfortunately, dietary intake was not strictly controlled for which increases the likelihood of confounding variables impacting results.

The length of this study, 12-week intervention, is a strength because it was long enough to illustrate how supplementation with dietary suggestions can impact a state of disease. The design of the study, a randomized double-blind placebo-controlled trial, is a strength because randomization increases the likelihood that treatment and control group are equivalent at baseline. A limitation of this study is the sample size being limited to a distinct group (NAFLD), this makes it difficult to extend to the general population. Another limitation is the lack fecal bacteria samples or inflammatory biomarkers to be able to assess and determine changes on the cellular level. These results support the effectiveness of probiotic and prebiotic supplementation as a method for therapeutic support in patients with NAFLD. However, further research utilizing biomarkers is necessary in order to determine the direct effect supplementation can have on biochemical parameters.

In 2011, the Clinic of the Institute of Nutrition in Russia performed a randomized, double-blind, placebo-controlled pilot study to evaluate the effectiveness of a reduced caloric diet with probiotic cheese in adults with metabolic syndrome (Sharafedtinov, Plotnikova, Alexeeva, Sentsova, Songisepp, Stsepetova, Smidt & Mikelsaar, 2011). The study included 40 hospitalized patients, ages 30 to 69 years old, diagnosed with metabolic syndrome specifically

obesity accompanied by hypertension (<130/85 mm Hg), and not currently receiving intensive treatment for other chronic diseases. Individuals were excluded from the study if they had a history of gastrointestinal disease, food allergy or acute infection, using antimicrobial agent in the last month, pregnant or breast-feeding. Throughout the three-week study, all participants followed a standard hypocaloric diet supplemented with 50 g/day of probiotic cheese or probiotic-free cheese (Sharafedintov et al., 2011). Anthropometric markers were measured to assess the effect inclusion of probiotic cheese had on patients. Blood pressure, blood samples, urine and fecal samples, and DNA extraction were evaluated to determine if there was an improvement at the molecular level.

Participants in the probiotic cheese group experienced a significant reduction in plasma triglyceride levels ($p = 0.041$) when compared to the control group ($p = 0.085$). A larger reduction in BMI was shown in the probiotic group versus the control group. There was also a significant increase in excretion of urinary putrescine in the probiotic group compared to the control ($p = 0.014$). Both groups saw a reduction in total cholesterol (probiotic $p < 0.001$, control $p = 0.004$) and in low-density lipoprotein levels (probiotic $p = 0.004$, control $p = 0.021$) (Sharafedintov et al., 2011). The authors concluded following a reduced caloric diet accompanied with a protein-rich full-fat cheese supported lowering blood glucose levels and stabilizing total cholesterol, low-density lipoproteins, and triglycerides (Sharafedintov et al., 2011). Unfortunately, despite a significant reduction in systolic blood pressure and diastolic blood pressure (BP); the BP-lowering drugs being taken by patients during the study were likely the reason.

The design of the study, a randomized double-blind placebo-controlled pilot trial, is a strength because randomization increases the likelihood that treatment and control group are

equivalent at baseline. Another strength is the use of biomarkers to efficiently assess changes at the molecular level, if they occurred. A limitation of this study was the sample size only being 40 participants, which makes it difficult to extend to the general population. Another limitation is the length of the study, three-weeks, which did not efficiently demonstrate any long term effects on weight or lab values. These results support the effectiveness of a hypocaloric diet supplemented with probiotic cheese as a non-medicine method to reduce symptoms of metabolic syndrome (Sharafedintov et al., 2011). However, further research is necessary to determine specific strains of probiotics to support long term weight loss and improvement in related lab values.

Analysis of Variations in Results

The previous studies applied a variety of interventions with differences in type, duration, and dosage of probiotics and/or prebiotics. Different trials targeted specific populations according to age, gender, disease state or BMI. The inclusion of varying types of brands, dosage, and lengths could have played a role in the large array of results produced. It remains unclear whether or not the incorporation of probiotic and/or prebiotic products truly impact the composition of the gut microbiome and eating behaviors.

Duration of Study

The duration of each study ranged from three weeks to 24 weeks. Three weeks is a sufficient amount of time to see a modification in gut bacteria; while 24 weeks provides an ample amount of time to collect a substantial amount of data to draw stronger conclusions related to weight reduction. The two studies that were shorter in length (three to four weeks) observed beneficial health results at the conclusion of the studies. Sharafedinov et al. (2015) established,

after three weeks, that a reduced caloric diet supplemented with probiotic cheese improved cholesterol related lab values in participants. In addition, Lawrence and Hyde (2017) recommended, for four weeks, a diet which increased vegetables and fermented foods while reducing processed items. Results from this study, supported a reduction in weight and improved mood related behaviors in participants. However, neither study examined the exclusive effects caused by a reduced calorie diet or probiotic supplement.

The three studies which were longer in length (12 to 24 weeks) critically analyzed favorable health outcomes associated with obesity. Behrouz et al. (2015) concluded, after 12 weeks, the use of a probiotic and prebiotic supplementation improved insulin metabolism and serum leptin in participants. Nicolucci et al. (2017) suggested, after 16 weeks, a prebiotic supplementation was effective in reducing weight in children for obesity prevention. Lastly, Sanchez et al. (2017) proposed, after 24 weeks, a reduced caloric diet with a probiotic supplement improved mood related behaviors and weight loss efficacy in participants. Despite significant results from all the previous studies, it is imperative to determine the validity and reliability of study results. It is vital to evaluate if biomarkers were utilized to support modification in gut bacteria occurred and strengthen results. Lastly, assessment of the results is necessary to determine the level of appropriateness for inclusion of probiotic and/or prebiotic supplementation in practice.

Type of Probiotic/Prebiotic

There was a variety of methods implemented to modify gut bacteria throughout these studies. Probiotic and prebiotic supplementation was supplemented with reduced caloric diets, along with fermented food items. Lawrence and Hyde (2017) emphasized the incorporation of

fermented foods such as; milk kefir, sauerkraut, tempeh, and miso versus capsule supplementation. Participants observed significant improvements in medical symptoms after the treatment period, however due to a lack of a control group and self-reporting the probably a placebo effect occurred is high. However, the use of fermented foods instead of supplementation supports the potential for dietary modifications to improve gut bacteria.

Sharafedinov et al. (2015) investigated a hypocaloric diet supplemented with probiotic cheese versus capsule supplementation. Participants in this study experienced improvement in BMI, metabolic syndrome symptoms, and arterial BP values. However, it was unclear how much the probiotic cheese alone impacted the weight loss and improvement in cholesterol and triglyceride lab values. Sanchez et al. (2017) examined a structured diet with a probiotic capsule supplementation versus solely food sources. Obese individuals included in this study saw improvements in mood and eating behaviors after the treatment period. Though, due to a calorie deficit diet and self-reporting methods it is difficult to accurately determine the impact supplementation truly had. Furthermore, individuals experienced weight loss which could be the underlying reason for mood improvement versus a probiotic supplement.

Behrouz et al. (2015) determined the incorporation of a probiotic capsule supplement with prebiotic powder had a favorable impact on glycemic parameters and leptin levels. Participants were provided with dietary modification and physical activity recommendations, making it uncertain whether or not these lifestyle interventions had a stronger impact on the outcomes. Finally, Nicolucci et al. (2017) suggested the use of prebiotic capsule supplement in overweight children for reduction in body fat. Children in the treatment group experienced weight loss and specific gut bacterial shifts. The study included children who were otherwise healthy, which supports the potential to use prebiotics for obesity prevention in the pediatric

population. However, future research is necessary within the pediatric population for application of appropriate preventative practice methods that utilize prebiotic supplementation. All the previous studies support use of a variety of types of probiotics and/or prebiotics, however further evaluation is necessary to determine an actual association between implementation, type, and beneficial outcome(s).

Dosage of Probiotic/Prebiotic

The dosing amounts between these five studies ranged from 0.626 g/day to 50 g/day, if dosage of the supplement was controlled. Lawrence and Hyde (2017) did not set a specific amount in grams for the fermented food items allowed within the controlled diet, instead guidelines specifying items to consume were provided. Compared to the other studies which included specific controlled numerical values throughout treatment periods; 50 g/day of probiotic cheese, five billion of five bacterial strains plus 16 g/day of prebiotic, 8 g/day of prebiotic, or 0.626 g/day of probiotic. Throughout the studies, dosage amount may have played an imperative role in varying outcomes observed. However, until a study evaluates different probiotic and/or prebiotic amounts within the same treatment period, it will remain unclear if distinct amounts are required or if a particular type will suffice. It is important to note, if the reviewed prior studies included a probiotic capsule supplement the capsule contained at least one Lactobacillus strain. This is one of the probiotic strains that past studies support may provide beneficial health outcomes in a variety of populations.

Future research is necessary to determine what the minimum amount of probiotic and/or prebiotic sources is necessary to support lasting improvements in gut bacteria. The evaluation and analysis of effectiveness in different dosages by age group is another area to study. This will

help narrow down the appropriate dose according to the different life stages individuals are in. Medications have the tendency to interact with each other; therefore, it is important to be aware of the potential for medications to impact effects of supplementation. Additionally, the array of eating patterns individuals follow needs to be taken into consideration since this may directly affect methods to alter gut bacteria as well.

Discussion

Researchers from the previous studies discussed daily diets that included a probiotic source produced a positive impact on mood, weight, and eating behaviors. Nicolucci et al. (2017) suggested an altered gut microbiome supported normalization of body weight gain based on total bacterial DNA analyses. Additionally, Sanchez et al. (2017) suggested dietary modifications with the addition of a probiotic supplement directly impacted the signals sent between the gut-brain axis based on changes in mood, cravings, and behavior questionnaires.

Merely one study reinforced the use of probiotic supplements for weight control in obese populations compared to five that produced inconclusive results. However, without the use of biomarkers for supportive evidence these results lacked validity. Mood related results were not as strong as the weight-loss outcomes because most of the studies relied on self-reported subjective questionnaires. Utilizing this technique weakens the study's results due to an increased subjectivity. This can increase the chance of imprecise data to be collected, recorded, and analyzed to support invalid study outcomes. Consequently, it is challenging to determine which influencing factor(s) impacted emotional, eating behavioral, and weight changes the most.

Conclusion

In conclusion, the previous articles provide inconclusive evidence if the incorporation of probiotic/prebiotic supplements along with dietary modifications could be connected to weight control via alterations of gut bacteria. A potential implication to practice is the recommendation of probiotic supplements for help with weight loss/control in an obese population. Initially, it would be imperative to recommend usage of the supplement in congruence with a dietary intervention to further support optimal outcomes. The previously mentioned studies examined a variety of probiotic and prebiotic supplements for analyzation of the occurrence for beneficial outcomes. New research has continued to support the existence of the gut-brain axis while investigating the gut microbiome composition.

Due to a lack of current strong evidence on probiotic and/or prebiotic supplements impact on mood and eating behaviors, there is a need for additional research to critically analyze the type, dosage, and length of intervention. Collecting this data to further evaluate the impact of alteration in gut bacteria composition on eating behaviors will aid in narrowing down future treatment recommendations within the field. By having future studies examine the impact different types of probiotic and prebiotic supplements can have on appetite control and mood regulation via gut bacteria, evidence in this area will strengthen and grow. Until then, practitioners should continue to recommend monitoring daily intake along with obtaining the essential nutrients for mood and weight regulation.

The gut microbiota is continually changing; thus, establishing if positive health outcomes can result from gut bacteria manipulation is the initial step. Then, development of a diverse set of interventions that influence gut bacteria, including diet modifications and probiotic/prebiotic

supplementation, is essential to fuel healthy gut bacteria for disease prevention. The research on the human gut microbiota has grown immensely over the last few years. The incorporation of advanced technology and associations for human health has played a big role in forward progression of the gut microbiome exploration; this will continue to grow and develop. One of the biggest challenges future researchers will face is attempting to determine whether these gut bacterial modifications are the cause of eating behavior changes and weight control.

Chapter 3: Proposal

One of the major challenges for healthcare professionals in the field of weight management is providing an effective long term weight reduction program. Since gut microbiota composition differs from individual to individual, recent studies have started to examine the effectiveness of manipulation of the microbiome to control eating behaviors. The purpose of this study is to examine if a daily probiotic and fiber supplement for three weeks impacts eating habits such as food cravings (intensity, frequency, self-control), fullness and hunger levels. Data from the intervention group and placebo group will be collected, analyzed, and compared to determine specific associations, if any.

Study Design and Objectives

This will be a randomized, placebo-controlled, blinded, cross-over 27-day study to investigate whether the daily uses of a probiotic supplement and/or a probiotic supplement plus fiber can affect eating habits (see Diagram 1). There will be an additional six days for two washout periods (lasting three days each) between phases to minimize attrition. According to Lawrence & Hyde (2017), a four-week study was a sufficient length of time to observe how dietary modifications impacted the participant's health outcomes. Additionally, Sanchez et al. (2017), carried out a 24-week study which revealed how a weight reducing program with probiotic supplementation affected mood and eating behaviors of obese individuals. Sharafedtinov et al. (2011), was unable to demonstrate potential long-term effects due to a shorter intervention study lasting three-weeks. Therefore, 27 days approximately four weeks, will provide enough time to see an effect of the probiotic supplement with fiber on eating

behavior according to prior studies ranging between three to 24 weeks in length, if an effect exists.

Several behavioral measures will be evaluated, through questionnaires, since gut health is directly associated with dietary intake. Questionnaires have been incorporated into prior studies for data collection due to their convenience of distribution and simplicity. Lawrence & Hyde (2017), assessed symptoms related to the digestive tract, the mind, and emotions through the Functional Medicine Medical Symptoms Questionnaire (MSQ). Sanchez et al. (2017), utilized a variety of questionnaires to assess multiple outcomes such as; Visual Analogue Scale for appetite sensations, Three-Factor Eating Questionnaire for eating behaviors, State-Trait Food Cravings for food cravings, and Beck Depression Inventory for mood components. Thus, two validated questionnaires, Food Cravings Questionnaire-Trait-Reduced and Visual Analog Scale for Appetite, will be used in this study to evaluate if food cravings and satiety levels were affected by probiotic supplementation with fiber.

The primary aim of this study is to identify whether incorporating a daily probiotic supplement or a daily probiotic supplement plus fiber for seven days has an immediate effect on eating behaviors (measured by self-reported questionnaires). The secondary aim is to evaluate the impact of a probiotic supplementation and fiber on weight change to determine if this could be a technique to implement for weight maintenance (measured by baseline and three-week total weight in pounds). Institutional review board (IRB) approval will be obtained prior to initiation of the study and data collection.

Recruitment and Sample Size

A convenience sampling method will be utilized to recruit subjects (after permission from the gym manager is obtained) by a flyer posted inside local gyms around the greater Milwaukee area. Recruiting within the gym setting will provide ample opportunity to include a variety of females interested in health and wellness. The study will be limited to females, ages 25-40 years, BMI classification of overweight or obese (>25, <35 BMI). Exclusion criteria are: male, allergy to probiotic or fiber products, comorbidities that are not suitable such as cardiovascular disease, fatty liver, hypothyroid, active colitis or immune-compromised conditions, smoker, and antibiotic treatment that could affect body weight and/or energy expenditure. Noted BMI range was chosen due to individuals with a BMI >35 are at risk for sleep apnea from obesity. Obstructive sleep apnea has been shown to impact insulin sensitivity which could hinder potential weight loss.

A target of 39 subjects will be recruited to allow for attrition, based on an online sample size calculator (http://www.sample-size.net/sample-size-study-paired-t-test/). The anticipated attrition rate is 20%, so the goal number of subjects was increased based on (n = 31/0.80 = 39). Subjects eligible for the study will be encouraged to be evaluated by their general practitioner prior to start of study. Informed consent will be obtained at that time.

Intervention

All subjects will provide a three-day food record and complete NHANES validated food frequency questionnaire (FFQ) prior to start of the study. These questionnaires will provide necessary data to determine the amount of fermented foods and fiber (over a set period of time) each participant is consuming at baseline. Subjects' total calorie, fermented foods, and fiber

consumption will be calculated by a registered dietitian nutritionist (RDN) at baseline. It is imperative to understand the consumption amount for fermented foods and fiber since participants will be following their regular diet throughout the study period. In addition, determining baseline eating patterns of the subjects is essential for analyzing and supporting tentative associations after the intervention period is completed to best isolate the independent variable as a cause of results.

Participants will be randomly assigned by hand to a sequence intervention group. Intervention groups last one week each: probiotic supplement, probiotic supplement plus fiber, and placebo; participants will complete these in different orders. There are a total of six different sequence intervention groups participants can be assigned. With a target of 39 participants, that puts approximately six individuals in each sequence intervention group (see Table 2).

The probiotic supplement is Culturelle Probiotics Digestive Health one tablet provides ten billion colony-forming units (CFUs) of Lactobacillus rhamnosus (LGG). This supplement was chosen because it contains no added sugar and was the most cost efficient product. The fiber supplement is Metamucil, chosen because it contains natural ingredients and no added sugar. Subjects will take approximately 18 grams (three teaspoons) of Metamucil mixed with eight ounces of water per one teaspoon three times per day. This provides an additional nine grams of dietary fiber to individuals' daily fiber intake from food items. Both supplements are brands commonly found in stores, therefore participants can gather additional information about these products. Lastly, the placebo tablet will be a sugar pill chosen to replicate the appearance of the probiotic supplement.

There will be an additional six days, two washout periods of three days between each intervention phase. Three days will be long enough to strongly rule out a carryover effect from one intervention to the next. Subjects will be encouraged to continue to follow their regular eating patterns and physical activity routines to minimize confounding variables. The order of interventions will vary to be able to test whether the order has an impact on outcome.

Data Collection

Participants' age, height, weight, and BMI will be measured at baseline and the end of the three-week intervention. Two validated questionnaires, Food Cravings Questionnaire-Trait-Reduced (FCQ-T-r) and Visual Analog Scale for Appetite (VAS) will be used to assess food cravings and satiety levels. Both of the questionnaires will be completed by all subjects three times throughout the trial; at baseline, at the start of each washout period, and completion of the study. Compliance with the probiotic supplement and fiber will be assessed by completion of a self-reported chart during each intervention phase. Participants will send their completed questionnaires and probiotic supplement with fiber tracking chart to the investigator via email.

Leptin and ghrelin hormones have been recognized to play a major role in hunger and satiety cues. Participants will have blood samples taken pre and post each intervention period to measure leptin and ghrelin levels. The blood samples before and after the interventions will suggest whether changes to food cravings and satiety are associated with inclusion of probiotic supplementation with fiber. Lastly, participants will complete a gut bacteria analysis swab via the uBiome kit pre and post each intervention. The fecal sample kits completed before and after the intervention periods will suggest whether changes to gut biome content are associated with changes in eating behavior.

Data Analysis/Statistical Tests

The scores from both questionnaires will be analyzed to determine if there is an association between probiotic supplement consumption and eating behaviors. A multivariable linear regression will be utilized to examine if associations between eating behaviors (food cravings and fullness level) and type of intervention (probiotic supplement or probiotic supplement with fiber) are present at baseline and completion of the 27-day study. To determine if an overall difference between eating behaviors (food cravings and fullness level) exists between intervention groups the ANOVA test will be performed. If a difference exists, the Bonferroni (a multiple comparison test) will be performed to determine which individual pairs of samples differ. To measure reliability of the statistical test, p-value and confidence interval will be calculated.

Chapter 4: Discussion

This study protocol was designed to investigate the impact of daily probiotic and prebiotics on eating behaviors via three intervention phases lasting seven days each in length. Outcomes will help determine whether prebiotics plus fiber and/or probiotics should be part of therapeutic treatment for weight management and preventative practices within the field.

Anticipated results

Weight Change

An average weight change of -0.68 kg (1.5 pounds) at completion of all three intervention phases is anticipated from the probiotic supplement and probiotic with fiber supplement groups. Sanchez et al. (2017), concluded that a specific diet containing fermented foods was associated with a positive impact on GI symptoms and weight control. Due to the weight loss observed in the Lactobacillus rhamnosus CGMCC1.3724 (LPR) supplementation intervention group (mean = -5.2 kg) compared to the control (mean = -2.5 kg), the expected weight loss with probiotic supplementation in this proposed study would feasibly be greater due to supportive results from prior studies (Sanchez et al., 2017).

Food Cravings

A reduction in the intensity of food cravings from four (categorized as often) to an average score of two (categorized as never) on the food cravings questionnaire is anticipated for the probiotic with fiber supplement group. A concurrent decrease in the frequency of food cravings from five (categorized as always) to an average score of three (categorized as sometimes) is expected for the probiotic with fiber supplement group from pre to post

intervention. This would support improvement in food cravings via reduction in frequency and intensity associated with use of the probiotic. Additionally, a 50% reduction in overall number of foods craved throughout the day is expected based on evidence from previous studies.

Satiety Levels

A reduction is anticipated in the duration of satiety in between meals from eight to nine (categorized as uncomfortable fullness) to an average score of six to seven (categorized as satisfied) reported on the visual analog scale (VAS). A concurrent decrease in participant's typical level of fullness throughout the day from eight to nine (categorized as uncomfortable fullness) to an average score of six to seven (categorized as satisfied) is also expected. These reductions would suggest that a probiotic supplement helps support greater satiety levels throughout the day.

Potential Criticism and Adaptations

There are numerous shortcomings that may interfere with the approval of this research proposal.

Gut Bacterial Strain versus Group Analysis

Since individual bacterial strain identification versus bacterial group analysis were not included, associations but not causation among the intervention and results can only be concluded. It would be yet to be determined whether gut manipulation is the reason for beneficial outcomes seen in eating behaviors and weight maintenance following the use of probiotics.

The participant acceptance rate with utilizing a fecal sample kit multiple times is unknown and may impact the statistical power if participants dropped out prior to completing the study. Including multiple fecal sample kits would increase the cost of the study and funding would be necessary for reducing the risk of a financial burden on the subjects. If the participant sample size is increased, 50% of the subjects could be randomly chosen to complete the fecal sample testing before and after the intervention. This would minimize the chances of a large drop-out rate, reduce the funding amount needed, and support stronger results.

Length of Study

The length of the study, 27- days total with seven days per treatment, raises additional concern on whether this time frame is long enough for gut bacteria modification and subsequent changes in food behaviors. The time frame is sufficient to observe immediate change in the gut microbiome, but it's uncertain if sustained changes in eating behavior would be observed. Previous studies have shown that the gut microbiome is manipulated quickly, however the length needed for changes in health outcomes is unclear. A potential solution to this problem would be to increase the length of each intervention and offer an incentive such as a free 20 minute nutrition counseling session with an accountability partner. This could entice subjects to complete the longer intervention periods and reduce risk for participant drop-out.

Recruitment

Another potential problem of this study is the method for recruiting the sample. Since it is a convenience sample, external validity is compromised. Further, the sample size only includes women. A potential solution to this problem would be to include men along with women in this study. Increasing the sample size would increase amount of data collected and

potentially strengthen associations. Convenience sampling could be extended to any gym within a 100 mile radius to support a larger and more diverse sample size.

Established Literature

Lastly, there has not been an abundance of research on manipulating the gut microbiome and monitoring health outcomes, making this more of a pilot study. A potential solution to this problem would be to revisit this proposal, in approximately five years, once more research on the gut microbiome has been completed. Allowing ample time for future studies to be carried out will increase the amount of evidence-based research on the gut microbiome and health outcomes and strengthen other results.

Potential Clinical Implications and Application

Future inclusion of probiotic and prebiotic supplementation for weight maintenance would be advised with existing medical nutrition recommendations (Sanchez et al., 2017; Behrouz et al., 2015). Probiotic supplementation would be recommended in addition to methods currently utilized within the field such as monitoring intake and including physical activity. The expectation of recommending a probiotic supplement with fiber would be to implement a comprehensive nutrition plan that is adaptable to individuals at all baseline levels. This inclusive nutrition plan would aim to improve a variety of risk factors, such as obesity or eating patterns with depleted levels of fiber, that directly impact an individual's health status. Probiotic supplementation with fiber may equally improve the target risk factors for disease development such as: weight, blood glucose levels, food cravings, and levels of satiety.

Furthermore, integration of probiotic supplementation within treatment plans would also support development and maintenance of a healthy gut microbiome. Healthy gut bacteria aids in

efficient digestion, strengthening the immune system, and keeps blood sugars balanced to support consistent energy levels. Theses health outcomes work toward lowering disease prevalence in the future. A reduction in the frequency of risk factors and disease occurrence may positively impact the overall wellness of individuals within the population.

Additionally, stronger practices that aim toward occurrence of obesity at younger ages is expected, based on a previous study with children (Nicolucci et al., 2017). Inclusion of a prebiotic supplement prompted gut bacterial shifts in children, in turn supporting a 3.1% decrease in body weight and 2.4% decrease in body fat (Nicolucci et al., 2017). The expectation would be to recommend supplementation for preventative practices at a variety of ages. The administration of probiotic and prebiotic supplements to prompt weight loss and improve laboratory values would be equally anticipated. Behrouz et al. (2015), suggested this intervention supports significant reductions in weight, body fat percentage, body mass index, and energy intake. This would target reducing the risk of childhood obesity and comorbidities.

Future Studies

Additional exploration of the strains, dose, and frequency of probiotics could possibly produce innovative dietary interventions to include within the medical nutrition therapy process for treatment of obesity and obesity-related diseases. Closely analyzing if individual strains such as Lactobacillus or Bifidobacterium or a combination of bacterial strains produce beneficial outcomes on eating behaviors will help create an efficient treatment plan for individuals. Once strains or combination of strains are identified, further analysis of the specific beneficial health outcomes such as weight loss and homeostasis within the microbiome is necessary. Prior studies suggest the incorporation of probiotics as a method for weight loss. It has been supported in

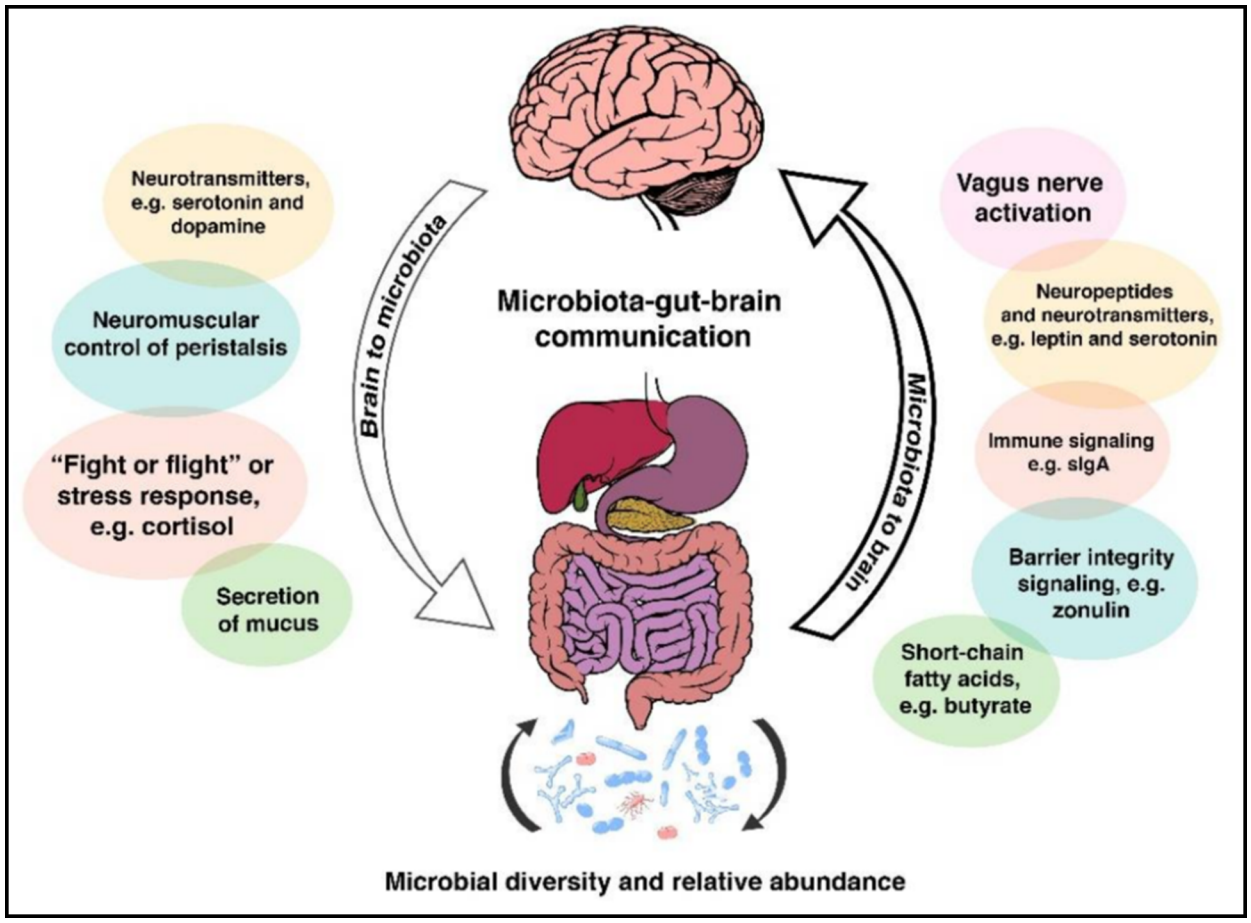
previous studies that reduction in weight is associated with less risk for developing obesity related disease such as, type 2 diabetes mellitus and heart disease. Therefore, obtaining further research in this area could support developing an effective way to reduce weight loss through probiotic supplementation may and decrease the prevalence of obesity along with common comorbidities.

It remains unclear the dose and duration for probiotic supplementation needed to produce favorable outcomes on eating behaviors. Further analysis of varying doses between different stages of life and genders are necessary for narrowing down amount recommended according to age group and/or gender. Evaluation of different amounts and durations is needed to conclude which combinations produce favorable long-term health benefits. Defining the specific lengths of probiotic supplement inclusion that support targeting specific states of disease and health circumstances to possibly lead to a reduction in future health risks and disease incidence.

Numerous aspects of the diet/probiotics and microbiome relationship require further research to identify their potential role in growth and maintenance of healthy gut bacteria. Microbial bacteria can feed or inhibit the growth of each other and are directly impacted by an individual's dietary habits. Closely analyzing these metabolic interactions among components of the microbiome will help establish how to trigger production of substances in the microbiome that improves growth in bacteria. Past studies support extreme diet changes, such as the ketogenic diet produce immediate changes in the composition of the gut microbiome. Investigation of diet changes at varying levels, such as extreme, mild, or moderate may help to link specific gut alterations with dietary patterns. Lastly, a normal microbiome enhances bioavailability of nutrients, however an altered microbiome may change the effect of food on the host. Additional research on varying microbiome environments and the impact on nutrient

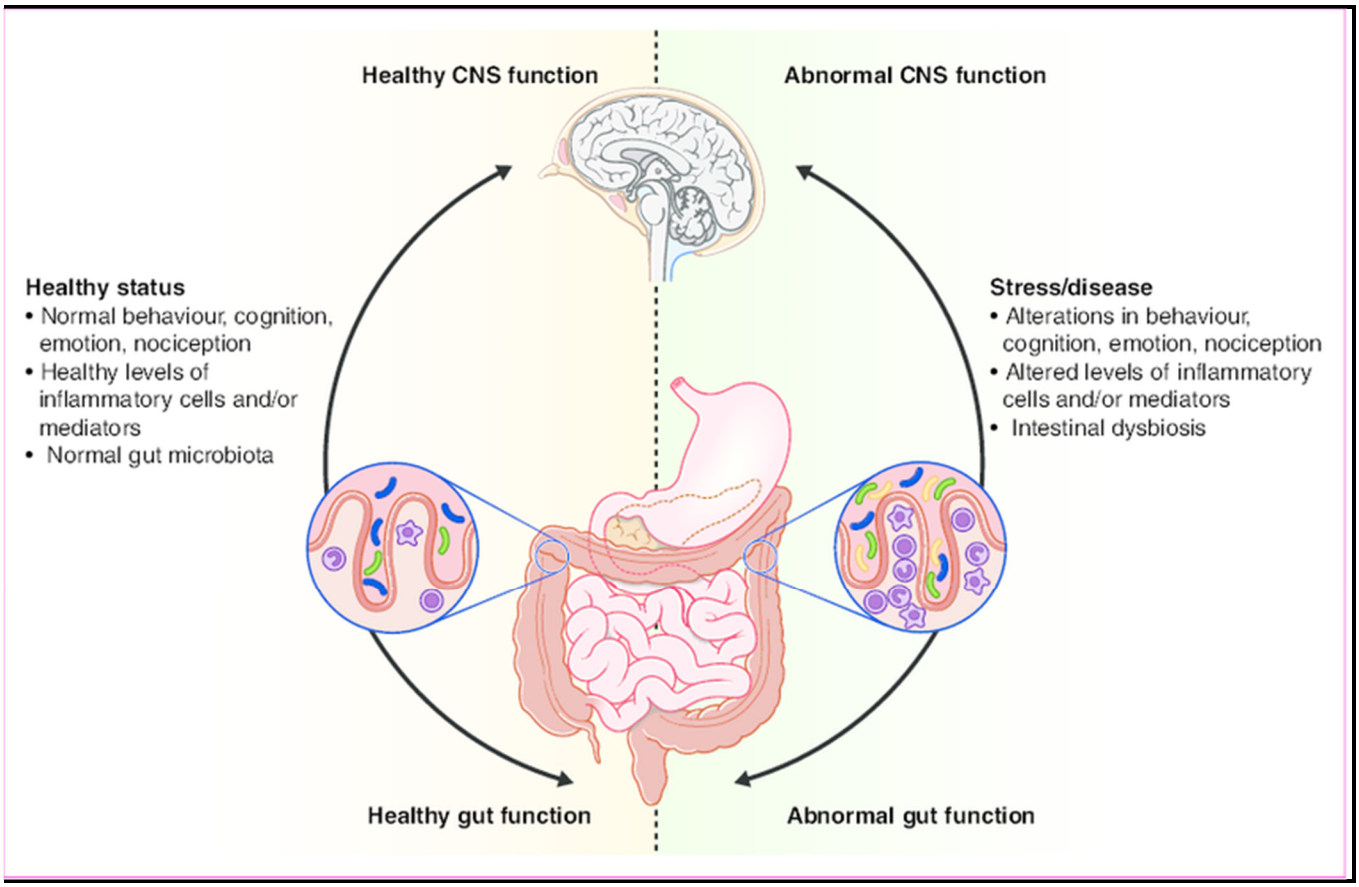
bioavailability may determine if an individual with an altered microbiome needs a diet consisting of higher nutrient quantity.

Figure 1



https://www.straightfromascientist.com/the-microbiota-gut-brain-axis-from-eubiosis-to-dysbiosis-and-back-useful-biomarkers-for-clinical-treatment/

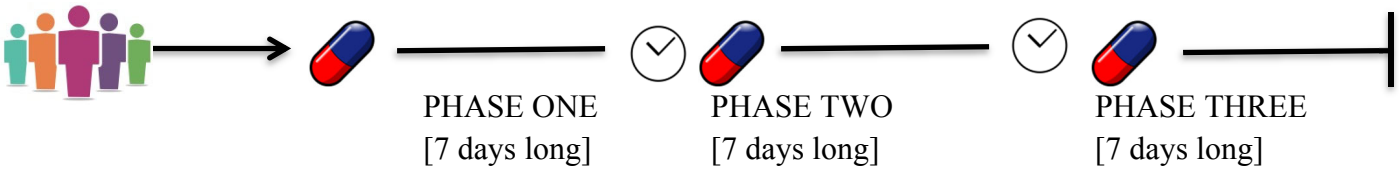
Figure 2


Healthy CNS function
Abnormal CNS function
Healthy status
• Normal behaviour, cognition, emotion, nociception
• Healthy levels of inflammatory cells and/or mediators
• Normal gut microbiota
Stress/disease
• Alterations in behaviour, cognition, emotion, nociception
• Altered levels of inflammatory cells and/or mediators
• Intestinal dysbiosis
Healthy gut function
Abnormal gut function

Gut microbiota - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/Impact-of-the-gut-microbiota-on-the-gut-brain-axis-in-health-and-disease-A-stable-gut_fig3_258198236 [accessed 30 Oct, 2018]

Diagram 1

Recruitment & Screen **Treatment [21 days + 6 days washout periods]**



PHASE ONE
[7 days long]

PHASE TWO
[7 days long]

PHASE THREE
[7 days long]

: Washout period [3 days in length]

Length of study: 27 days total

Table 1

Current Probiotic Supplements

Name	Cost	Contents	Purported Benefits
Complete Probiotic Platinum	$49.00/30 capsules	51 billion CFUs with 11 strains, prebiotic fiber	Promotes a healthy bacterial balance in the digestive tract with high-quality strains.
PRO-45	$34.95/30 capsules	45 billion CFUs with 11 top strains, prebiotic fiber	Balances the gut and improves digestive health.
Ultimate Flora Extra Care Probiotic 50 Billion	$39.99/30 capsules	50 CFUs with 12 strains, fiber	Restores the body's good, natural flora, relives occasional digestive discomfort and promotes digestive and immune health.
Culturelle Digestive Health Probiotic	$33.98/80 capsules	10 billion CFUs of Lactobacillus GG	Keeps reduce digestive upset and helps the digestive system work better.
Ultra-30 Probiotics	$28.95/60 capsules	30 billion CFUs with 18 strains	Boosts the immune system, promotes healthy digestion, and helps alleviate occasional constipation.
Gut Instinct	$25.00/30 capsules	25 billion CFUs with 10 strains	Benefits your skin, health, and beauty. Keeps the digestive tract healthy.
Now Foods Probiotic-10	$14.20/50 capsules	25 billion CFUs with 10 strains	Supports immune health and healthy intestinal flora.
Probiotic Complex: Daily Need	$24.99/30 capsules	25 billion CFUs, prebiotic	Digestive and immune support.
Dr. Formulated Probiotics: Once Daily Women's	$29.39/30 capsules	50 billion CFUs with 16 strains	Supports women's health, immune system and digestive health.
Raw Probiotics Ultimate Care	$64.99/30 capsules	100 billion CFUs with 34 raw strains, protein digesting enzymes	Achieve balance, helps repopulate the gastrointestinal tract with good bacteria.

Table 2

Potential Sequences for Intervention Phases

Groups	Sequences
A: Probiotic supplement	**ABC**
B: Probiotic supplement + fiber	**ACB**
C: Placebo	**BAC**
	BCA
	CAB
	CBA

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Appendix A

Mount Mary University
Institutional Review Board (IRB)
for the Protection of Human Subjects

Application for IRB Review

DATA COLLECTION CANNOT BEGIN
UNTIL THE IRB HAS APPROVED THIS PROJECT

Directions:

- Faculty and student researchers, as well as student research advisors, should **read all relevant information on the University IRB page in My Mount Mary before initiating an application**. This includes full knowledge of the US Department of Health and Human Services Code of Federal Regulations Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html
- All applicants must verify completion of Human Subjects Training. See http://www.citiprogram.org
- The IRB application must be filed and approved by the IRB **prior** to any Mount Mary University faculty, staff, or student (undergraduate or graduate), initiating a research project/study.
- If there is a cooperating institution, attach a copy of their IRB approval.
- In the case of a student research project, the student may complete the IRB application but the student's research advisor must sign and submit the application to the IRB for approval. It is the responsibility of the faculty research advisor to ensure that student applications and all attachments (e.g. informed consent forms and survey instruments) are in their final edited form. Even though a student research project may qualify as **exempt** from full IRB review, the research advisor may request the student to complete and submit a full IRB application.
- Complete this application using your word processing program (ex. Word), then print it out and obtain signatures from all investigators and advisors. (**Handwritten applications will not be accepted**.) For your benefit, save the completed application on your computer in case it needs to be revised and resubmitted.
- This is a professional document; please check spelling, grammar and punctuation.
- Submit a hard copy of the completed application with required signatures and attachments to Maureen Leonard, IRB Chair, Sciences Department. **(Emailed applications will not be accepted.)**
- Allow a **minimum of 10 working days** to process your application. Make sure this time frame is accounted for when considering initiation of data collection and due dates for student projects.
- For class projects you must submit IRB applications to the IRB Chair by October 31st of the fall semester and March 31st for the spring semester. For summer classes, please consult with the IRB Chair.
- Upon receipt of the IRB letter of approval, data collection may begin.

I. Required Documentation **(No action will be taken without these attachments.)**

Are the following attached to the IRB application?

Consent application	☒ Yes	Applications should include explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix.A.
Questionnaire/Survey Instrument(s)	☒ Yes	If survey is being conducted verbally, a copy of the introductory comments and survey questions being asked must be attached to this application. If survey includes focus group questions, a complete list of the question should be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice.
Verification of Human Subjects Training	☒ Yes	Copy of transcript, certificate or other evidence
Copy of cooperating institution's IRB approval.	☐ Yes	Not required if there is no cooperating institution. .

II. Investigator(s):

Name: Mandy Mindin Phone: 414-975-9622

Affiliation with Mount Mary University (e.g. faculty, student, etc): Dietetics Graduate Student

Email: mindina@mtmary.edu

Signature: Mandy Mindin Date:

Name: Phone:

Affiliation with Mount Mary University:

Email:

Signature: _____ Date:

If student, list Research Advisor and complete Section II. Research Advisor must provide requested information and verify.

Department: Dietetics

Research Advisor's Name: Dr. Megan Baumler

Email: baumlerm@mtmary.edu Phone: 608-669-4234

Research Advisor: Have you completed Human Subject's Training? ☒ Yes ☐ No

Research advisor's signature indicates responsibility for student compliance with all IRB requirements.

Signature: _____ Date:

Research Advisor

III. Project Description

Instructions: Briefly describe the proposed project including the sample and methodology (e.g. human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):

The purpose of this study is to examine if a daily probiotic and fiber supplement for three weeks impacts eating habits such as food cravings (intensity, frequency, self-control), fullness and hunger levels. The eating habits previously listed will be measured by a validated food cravings questionnaire and visual analog scale for appetite. The probiotic supplement is Culturelle Probiotics Digestive Health. This supplement was chosen because it contains no added sugar and was the most cost efficient product. The fiber supplement is Fiber Choice, chosen because it contains natural ingredients and no added sugar.

2) Relevance to practice/body of knowledge:

Past studies have supported daily incorporation of specific probiotic strains had an impact on mood and eating behaviors. Previous research has also suggested incorporation of probiotic supplementation could have an influence on weight loss. This study will potentially be able to provide further support on the use of probiotic and fiber supplementation for weight control in overweight or obese populations. Dietitians could use this information to provide guidance and recommendations for probiotic supplements to clients and patients.

3) Describe the research design (e.g. subject/participant selection and assignment, design, intervention, data analysis):

Design and Subject Selection: The proposed study is a randomized, placebo-controlled, blinded, crossover three week study. The goal number of subjects is 30 based on an online sample size calculator (http://www.sample-size.net/sample-size-study-paired-t-test/). The anticipated attrition rate is 20%, so the goal number of subjects was increased based on (n = 30 x 0.2 = 6; 30 + 6 = 36). Inclusion criteria are: female, ages 25-40 years, BMI classification of overweight or obese. Exclusion criteria are: male, allergy to probiotic or fiber products, comorbidities that are not suitable such as cardiovascular disease, fatty liver, active colitis or immune-compromised conditions, smoking, and antibiotic treatment that could affect body weight and/or energy expenditure. Subjects will be recruited (after permission from the gym manager is obtained) by a flyer posted inside local gyms around the greater Milwaukee area. Three weeks with six days for two washout periods will minimize attrition, yet is long enough to see an effect of the supplement on eating behavior according to previous four week studies, if an effect exists. Compliance of the supplements will be assessed by completion of a self-reported chart during each intervention phase.

Assignment to Groups: Participants will be randomly assigned by hand to a sequence intervention group. Intervention groups last one week each: probiotic supplement, probiotic supplement + fiber, and placebo. There will be a washout period of three days between each intervention phase. Three days will be long enough to strongly rule out a carryover effect from one intervention to the next. Subjects will be encouraged to continue to follow their regular eating patterns and physical activity routines to minimize confounding variables. The order of interventions will vary to be able to test whether the order has an impact on outcome.

Data Analysis: The scores from both questionnaires will be analyzed to determine if there is an association between probiotic supplement consumption and eating behaviors. A multivariable linear regression will be utilized to examine if associations between variables are present at baseline and completion of the three week study. To determine if an overall difference exists between intervention groups the ANOVA test will be performed. If a difference exists, the Bonferroni (a multiple comparison test) will be performed to determine which individual pairs of samples differ. To measure reliability of the statistical test, p-value and confidence interval will be calculated.

4) What measurement/data collection tools are being used?

Two validated questionnaires will be used to assess food cravings and satiety levels. These are included in this application. The questionnaires will be completed by all subjects at baseline, at the start of each washout period, and completion of the study. A reminder email will be sent to all participants at the end of each treatment phase to complete the questionnaires. There will also be a chart used to track compliance of the probiotic and fiber supplements.

Is the proposed project "research" as defined by Institutional Review Board requirements?

- Research is defined as a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.
- A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information.

Does the research involve human subjects or official records about human subjects?

☒ Yes
☐ No

***If NO STOP here and SUBMIT application*.**

If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:

☒ Yes
☐ No

If the YES box is CHECKED, proceed to SECTION IV.

***If the NO box is CHECKED, STOP here and SUBMIT application*.**

IV. Exemptions

Are you requesting exemption from IRB review in one of the federally approved categories? If yes, please reference OHRP website http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html and continue with application.

1) Does the research meet the criteria for exempt category 1 (education)? [45 CFR 46.101 (b) (1)]

Is the research conducted in established or commonly accepted educational settings (e.g. schools, Universities or other sites where educational activities regularly occur)?
☐ Yes
☒ No

Does the research study involve only normal education practices (e.g. instructional strategies, techniques, curricula, or classroom management techniques)?
☐ Yes
☒ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

2) Does the research meet the criteria for exempt category 2 (specific procedures)? [45 CFR 46.101 (b) (2)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior?
☐ Yes
☒ No

Is the information obtained recorded in such a manner that human subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)

☒ Yes
☐ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

3) Does the research meet the criteria for exempt category 3 (public officials)? [45 CFR 46.101 (b) (3)]

Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior?

☐ Yes
☒ No

Are the human subjects elected or appointed public officials or candidates for public office? **If no, proceed to Category 4.**

☐ Yes
☒ No

Does any federal statute require without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter? (See Appendix B)

☐ Yes
☐ No

*If **all** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application*

4) Does the research meet the criteria for exempt category 4 (existing data/specimens)? [45 CFR 46.101 (b) (4)]

Does the research involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens?

☐ Yes
☒ No

☒ Yes

Will the information be recorded by the investigator in such a manner that the subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B)

☐ No

*If **both** questions are answered **yes,** stop here, proceed to **Section I Required Documentation**, and **submit** application.*

5) Does the research meet the criteria for exempt category 5 (federal program research)? [45 CFR 46.101 (b) (5)]

Does the research involve studying, evaluating or examining federal public benefit or service programs?	☐ Yes ☒ No
Is the research conducted through a federal agency?	☐ Yes ☒ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

6) Does the research meet the criteria for exempt category 6 (taste and food quality)? [45 CFR 46.101 (b) (6)]

Does the research involve a taste and food quality evaluation or consumer acceptance study?	☐ Yes ☒ No
Does the food consumed contain no additives, or a limited amount of food additives at or below a level approved by the FDA or EPA or the Food Safety and Inspection Service of the U.S. Department of Agriculture	☐ Yes ☒ No

*If **both** questions are answered **yes**, stop here, proceed to **Section I Required Documentation**, and **submit** application.*

If no exemptions apply, continue with application.

V. Additional Project Information

1) What human subjects training has the researcher completed (e.g. course work, online certification)? CITI Human Subjects Research Online Training

2) What process is used for obtaining informed consent (attach the informed consent application)? See Appendix for consent application.

Informed consent form will be distributed and explained to each participant prior to starting the study.

3) Does the research include special populations?

Minors under 18 years of age?	☐ Yes	☒ No
Persons legally incompetent?	☐ Yes	☒ No

Prisoners?	☐ Yes	☒ No
Pregnant women, if affected by research?	☐ Yes	☒ No
Persons institutionalized?	☐ Yes	☒ No
Persons mentally incapacitated?	☐ Yes	☒ No

4) If **YES,** describe additional precautions included in the research procedures.

N/A

5) Does the research involve any of the following procedures?

False or misleading information to subjects?	☐ Yes	☒ No
Withholds information such that their informed consent might be questioned?	☐ Yes	☒ No
Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects?	☐ Yes	☒ No

6) If **YES**, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

N/A

7) Does the research involve measurement in any of the following areas?

Sexual behaviors?	☐ Yes	☒ No
Drug use?	☐ Yes	☒ No
Illegal conduct?	☐ Yes	☒ No
Use of alcohol?	☐ Yes	☒ No

8) If **YES**, describe additional precautions included in the research procedures.

N/A

9) Are any portions of the research being conducted online?

Survey posted on a website?	☐ Yes	☒ No	If yes, assure anonymity
URL for survey includes information that could identify participants?	☐ Yes	☒ No	If yes, assure anonymity
Invitation to participate sent by email?	☒ Yes	☐ No	If yes, assure anonymity
Items use drop-down box?	☐ Yes	☒ No	If yes, assure that items allow choice of "no response"

10) If **YES**, describe additional procedures.

An initial email (see attached) will be sent to potential participating women who fit the inclusion criteria. This will be sent out once a consent form is completed with their contact information. This email will include details on the study such as the specific brand to purchase for probiotic and fiber supplements, where to purchase the supplements, and validated charts for self-reporting. I will be utilizing the "BCC" option in order to ensure identities are kept confidential from other participants and no further contact is possible between participants. Once the study is underway, participants will have the option to contact me individually via email if any questions or concerns arise.

11) Describe the methods used to ensure confidentiality of data obtained.

Participants' names will not be kept on any documents except a participant key for the randomization process. Recorded craving and satiety charts will be destroyed after data analysis is complete. The name or other identifying information of participants will not be reported in any publications. The key linking the participants' name to the data will be destroyed after data analysis is complete.

Risks and Benefits

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk).

There is a possibility for dehydration to occur if adequate water is not being consumed with the fiber supplement. There is also the potential for mild side effects, if they occur, such as gas or bloating. In order to minimize the chance of these risks occurring, I will state in the initial email to participants a sufficient amount of water is necessary while they are taking the fiber supplement, and will remind them prior to the start of the study. The email will also explain the importance for reading the labels on each supplement container to ensure that if an allergic reaction occurs, proper steps will be taken to stop the reaction.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)

Research has supported the use of probiotic supplementation may improve nutrient absorption, enhance cognitive function, regulate mood, optimize digestion, and reduce systemic inflammation. An adequate amount of fiber has been supported to normalize bowel movements, control blood sugar levels, and support satiety cues such as decreasing hunger and keeping the stomach feeling full due to a longer digestion period.

Appendix A: Required Elements of Informed Consent

Informed consent is the process of communicating to a prospective participant, in easy-to-understand language (usually sixth- to eighth-grade level), all that he or she needs to know about participating in a research project, and then obtaining the prospective participant's agreement to participate. The following ten elements of consent are widely recognized and, except under certain specific conditions, **must be included in all consent processes and forms**:

1. An explanation of the study, including goals, procedure, and a statement that the study is research.
2. A description of what participants are expected to do and expected length of participation.
3. A description of any likely risks or discomforts for the participants. Potential harm should be explained in language that participants can understand and that relate to everyday life.
4. A description of any likely benefits to the participant or to others.
5. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant.
6. A statement describing the level of privacy assured for collected information (anonymous, confidential) and how private information and information security will be managed.
7. An explanation of whom to contact for answers to questions about the research. When a Mount Mary student is the principal investigator, the name and phone number of a supervising faculty member is required.
8. An explanation of whom to contact for concerns about the participant's privacy and rights, which for Mount Mary University is its IRB Chair.
9. For research involving more than minimal risk, a statement describing any compensation for injuries and contact information. (Minimal risk is a risk of harm to the participant that is no greater than the risk encountered in normal, day-to-day activities or during routine physical or psychological examinations.)
10. A statement that research participation is voluntary and the participant may withdraw from participation at any time, without penalty or loss of benefits to which the participant is otherwise entitled. If the participant is a patient or client receiving medical, psychological, counseling, or other treatment services, there should be a statement that withdrawal from the study will not jeopardize or otherwise affect any treatment or services the

participant is currently receiving or may receive in the future. Participants also should be told whether their data will be destroyed should they withdraw from the study. If a survey instrument or interview questions are used and some questions deal with sensitive issues, the participants should be told they may refuse to answer individual questions.

Appendix B: IRB De-Identification Standard for Information

Protecting the privacy of research participants is a general concern in the vast majority of research projects. The degree to which privacy needs to be ensured or maintained depends on the nature of the particular research, its setting, and the research participants. Researchers share a general obligation to design their research to reduce the risks of disclosure of collected information about individual research participants. Thus, the present standard for de-identification of information is useful as a guide to protecting privacy even when it is not required or fully required. In this regard, the researcher should consider the following question when collecting and handling data.

Does the information I am accessing, recording, and/or disclosing contain identifiers? Simple access to information may be without concern, for example when the researcher is an employee who routinely handles the records in carrying out his or her position. But, the presence of identifiers in any **recorded or disclosed** information in the research means the information is not anonymous and so does not meet the IRB de-identification standard, which in some cases may also disqualify the research from exemption from IRB review. The IRB de-identification standard includes all 18 direct identifiers specified in the HIPAA Privacy Rule de-identification standard—*45 CFR 164.514(b)*. Below are listed specific direct and indirect identifiers that lead to information not being anonymous.

Identifiers: Direct; Indirect

One way to distinguish between information that is truly anonymous and information that is simply being kept confidential is to determine whether the data set contains direct or indirect identifiers. Information in a data set with either direct or indirect identifiers is not anonymous.

Direct Identifiers include:

- Names
- Addresses
- Telephone and fax numbers
- Email addresses, IP addresses, and URLs
- Social Security numbers
- Medical record numbers
- Account numbers, such as those associated with bank accounts or health plans
- License or certificate numbers, including driver's license numbers
- License plate numbers and other vehicle identifiers
- Fingerprints, voiceprints, or full-face photographic images
- Other unique characteristics or identification numbers (example student ID numbers)

Indirect Identifiers can be combined with publicly available information to identify individuals. The determination of indirect identifiers depends on the nature of the research participants. For example, in a study of residents of the state of Wisconsin, the information that someone graduated from one of the UW system schools probably would not be a unique identifier. However, in a study of small business leaders in Racine, WI, the same information might well apply to only one individual. In general, if any single variable in a data set applies to fewer than five participants, it is considered a potential indirect identifier.

Examples of indirect identifiers include:

- Detailed geographical information, such as state, county, or census tract of residence
- Organizations to which participants belong
- Educational institutions from which participants graduated
- Exact occupations

- Places where participants grew up
- Many dates, e.g. birth dates, hospital admission dates, high school or University graduation dates, etc.
- Detailed income information
- Offices or posts held by participants.

Appendix B

Consent Form for Participation in a Research Study
Mount Mary University

A Randomized Crossover Trial Evaluating the Impact of Probiotic and Fiber Supplementation on Eating Habits

Description of the research and your participation

You are invited to participate in a research project being conducted by Mandy Mindin, a dietetics graduate student at Mount Mary University. The purpose of this research is to examine if a probiotic supplement with fiber can positively impact eating habits.

Participants' expectations

If you decide to participate, you will be asked to rate your food cravings and satiety (fullness) levels on questionnaires throughout the three week study period. You will also be expected to consume a probiotic supplement and fiber supplement during the intervention phases (one week for each product). Participants will be in charge of purchasing the specified probiotic and fiber supplements on their own.

Risks

- In addition to your time and inconvenience, there is a possibility for dehydration to occur if adequate water is not being consumed with the fiber supplement.
- There is the potential for allergic reactions from either or both supplements.

Benefits

- Research has supported probiotics may improve nutrient absorption, enhance cognitive function, regulate mood, optimize digestion, and reduce systemic inflammation.
- An adequate amount of fiber has been supported to normalize bowel movements, control blood sugar levels, and support satiety cues.

Compensation

For purchasing the supplements and completing the study, each participant will be given two 15 minute nutrition education sessions on general weight loss tips and healthy eating methods.

Confidentiality

Your name will not be kept on any documents except a participant key for the randomization process. Recorded craving and satiety charts will be destroyed after data analysis is complete (by or before July 2017). Supervising faculty member, Dr. Megan Baumler will be overseeing this process. Your name or other identifying information will not be reported in any publications. The key linking your name to the data will be destroyed after data analysis is complete.

Voluntary Participation

Participation is voluntary. If you choose to take part in this study, you may stop at any time during the study. Stopping the study will alter the compensation you will receive.

Contact Information

If you have any question about this study, please contact me by email (mindina@mtmary.edu). Any additional questions concerning the research process, please contact Dr. Megan Baumler at 608-669-4234 (or email baumlerm@mtmary.edu).

Consent

I have read this consent form and have been given the opportunity to ask questions. I give my consent to participate in this study.

Participant's signature_____ Date:_____

A copy of this consent form should be given to you.

Appendix C

Food Cravings Questionnaire—Trait—Reduced

1. When I crave something I know I won't be able to stop once I start.
2. If I eat what I am craving, I often lost control and eat too much.
3. Food cravings invariably make me think of ways to get what I want to eat.
4. I feel like I have food on my mind all the time.
5. I find myself preoccupied with food.
6. Whenever I have cravings, I find myself making plans to eat.
7. I crave foods when I feel bored, angry, or sad.
8. I have no will power to resist my food crave.
9. Once I start eating, I have trouble stopping.
10. I can't stop thinking about eating no matter how hard I try.
11. If I give in to a food craving, all control is lost.
12. Whenever I have a food craving, I keep on thinking about eating until I actually eat the food.
13. If I am craving something, thoughts of eating it consume me.
14. My emotions often make me want to eat.
15. It is hard for me to resist the temptation to eat appetizing foods that are in my reach.

1	2	3	4	5
Never	Rarely	Sometimes	Often	Always

Original item numbers refer to the 39-item version as displayed in Cepeda-Benito et al. (2000a,b), Meule et al. (2012a).

Appendix D

Visual Analog Scale (VAS) for Appetite

How does your stomach feel after you eat?

Starving
_____Overstuffed, Disgusted

0 1 2 3 4 5 6 7 8 9 10

Comments:

What is your hunger at right now?

Starving _____Overstuffed, Disgusted

0 1 2 3 4 5 6 7 8 9 10

Comments:

What is your typical or average fullness?

Starving
_____Overstuffed, Disgusted

0 1 2 3 4 5 6 7 8 9 10

Comments:

0 -1	2 - 3	4 – 5	6 - 7	8 - 9	10
Starving	Mild hunger	Desire to eat	Satisfied	Uncomfortable fullness	Overstuffed, disgusted

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