The Gut Microbiota and Fermented Food: A Nutraceutical Influence on Alzheimer’s Disease

A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science in Molecular Microbiology and Immunology and the Honors College

By

Madison Sommer

Dr. William Courchesne/Thesis Advisor

May 2020
We recommend that the thesis prepared under our supervision by

Madison C. Sommer

entitled

The Gut Microbiota and Fermented Food: a Nutraceutical Influence on Alzheimer’s Disease

be accepted in partial fulfillment of the requirements for the degree of

MOLECULAR MICROBIOLOGY AND IMMUNOLOGY, BACHELOR OF SCIENCE

_________________________  ______________________
William Courchesne Ph.D., Thesis Advisor

Matthew Means, P.S., Director, Honors Program

May 2020
ABSTRACT

Alzheimer’s disease (AD) is a neurodegenerative disease responsible for most cases of dementia in elderly people. The exact cause of AD is unknown. Many physiological dysfunctions can be related to AD including neuronal decay in the brain, gut dysbiosis, and impaired hormone and oxidation pathways. Of particular interest is the gut dysbiosis experienced by people with AD. People with AD have a decreased abundance of Firmicutes and Actinobacteria, and increased abundance of Bacteroidetes compared to people without AD. On a genus level *Bifidobacterium* (Acinetobacter) is decreased. *Bifidobacterium* are commonly found in fermented foods such as natto, kefir, kombucha, sauerkraut, and tempeh. Foods fermented with *Bifidobacterium* tend to also be populated with lactic acid bacteria (*Lactobacillus, Lactococcus*, and *Leuconostoc*). Fermented food has additional benefits beyond being simple probiotics. In addition to their probiotic effects, when consumed for long periods of time, fermented foods possess enzymatic, anti-inflammatory, and antioxidant activities that have been shown to remedy the neurological effects of AD. Since some fermented foods (natto, kimchi, tempeh, etc.) have been shown to be probiotic, they could have similar effects.
ACKNOWLEDGEMENTS

I would like to thank all the people who have helped me along this journey. Your support and advice have been instrumental in getting me through this process. First, I would like to thank the Honors College for giving me this opportunity to explore a field in my area of study. I would also like to thank the Honors College for everything they have done for me over the past four years. I have valued the classes and functions put on by the Honors College for the sense of community they created. I would also like to thank Melissa Gunby and Dr. Johnson for helping me work through ideas and organizational problems. Finally, I would like to thank my mentor Dr. Courchesne for being supportive and giving me guidance over the last years not only on this thesis but in my general education in the MMI department. Without the support of the people around me I would have been lost. Thank you.
CONTENTS

I. Background on fermented food, the gut microbiota, and the gut-brain axis ............ 1
   A. Fermented Food .......................................................................................................... 1
   B. Gut Microbiota ........................................................................................................... 3
   C. The Gut-Brain Axis ..................................................................................................... 8
II. Fermented Food and the Gut Microbiota ..................................................................... 18
III. The Gut Microbiota & Alzheimer's disease ................................................................. 23
IV. AD: Oxidation, GABAergic pathway, and the HPA .................................................... 29
CONCLUSION .................................................................................................................. 31
REFERENCES .................................................................................................................. 32
I. Background on fermented food, the gut microbiota, and the gut-brain axis

A. Fermented Food

As human beings moved apart from one another, they encountered different food and different microorganisms fostering a wide variety of cultural adaptations of fermented food. Almost every culture around the world has fermented food incorporated in its diet whether it is kimchi in Korea, natto in Japan, or tempeh in Indonesia. Some fermented foods have become universal staples including yogurt, sauerkraut, and wine. One of the oldest forms of food preservation, still used today, fermentation allows people to preserve food while at the same time improving its flavor and making the food safer and easier to eat. In ancient times, fermentation of fruit and vegetables, dairy, meat, and grain was used to preserve their nutritive value and prevent spoilage. Yogurt, cheese, wine, salami, sauerkraut, bread and many more foods produced with the help of microbes last much longer than their raw ingredients (milk, fruit, raw beef, cabbage, grain, etc.) (1). For example, the natural microbiota of meat consists of putrefying spoilage bacteria. Fermentation with lactic acid bacteria prevents spoilage and creates a whole new flavorful product (2). Despite the popularity of canning, freezing, chemical preservatives, and irradiation, fermentation remains a staple and reliable form of food preservation. Fermentation is not only used for preservation, but in the general processing of food in order to improve access and make the food edible. For example, foods, like soy sauce, coffee, and chocolate may not contain living microorganisms, but microbes are still used in the processing of these foods in order to produce the familiar food product from its inedible source (1).
Fermented foods are foods and beverages produced through controlled microbial growth resulting in the conversion of food components through enzymatic actions (3). Food fermentations are bioprocesses that change food properties. Fermentation occurs in the absence of oxygen, under anaerobic conditions, and is primarily related to the incomplete breakdown of carbohydrates in order to generate energy for the bacteria. In the process of food breakdown for microbial energy, a variety of metabolites are produced that contribute to the flavor, texture, and health properties seen in fermented foods (2). Foods can ferment naturally using wild ferments present in the natural environment of the food, or through the addition of starter cultures (3).

Many cultures that have long since incorporated fermented foods into their diets have boasted their health benefits for generations (1). Fermented food can exert health benefits through multiple mechanisms including increased bioavailability, generation of additional nutrients and health promoting compounds, removal of toxins and anti-nutrients, and probiotic functions (1, 3). Pre-digestion as a result of fermentation makes B vitamins more bioavailable, increasing the concentration of B vitamins in the fermented food compared to the unfermented food. The breakdown of phytochemicals in cabbage are shown to produce anticarcinogenic compounds. Fermentation of some food products removes anti-nutrients or toxins, sometimes turning them into nutrients or just making the food safe to eat. For example, raw cassava is inedible due to high concentrations of cyanide, however after peeling and fermentation they become detoxified (1).

Consumption of some fermented foods such as natto, kimchi, and tempeh has been shown to alter the abundance of some microbes in the gut microbiota, demonstrating the probiotic effect of fermented food (3).
The probiotic function of fermented food has the added benefit of introducing enzymes associated with certain microorganisms. For example, people with lactose intolerance may find it more tolerable to consume fermented dairy products such as yogurt due to the presence of lactase, an enzyme that breaks down lactose and is introduced to the gut by lactic acid bacteria (2). Another example is natto, a fermented soy product from Japan, which contains the enzyme nattokinase. Nattokinase has been shown to have fibrinolytic activity. This enzymatic activity is key in controlling diseases like hypertension, atherosclerosis, coronary artery disease, and stroke. Research has also found nattokinase to be capable of degrading amyloid fibrils which accumulate and are to blame for some symptoms of Alzheimer’s disease (1).

Together, the health benefits of fermented food can influence a variety of diseases. The probiotic ability of fermented food could potentially serve as a means of altering dysfunctional gut microbiotas through the introduction of beneficial organisms therefore influencing disease states by restoring the gut to its healthy form.

B. Gut Microbiota

Every day, people are making new discoveries about the human body. A recent area of interest of the past few decades has been the existence of microbes inside and outside the body. There are more microbial cells living in the body than human cells. Populations of microbes exist in specific regions throughout the body and are referred to as microbiomes. A microbiome constitutes the entire genetic material that makes up an organism. There are multiple microbiomes that make up the human microbiome as a whole and each is unique. For example, an individual’s gut microbiome is distinct from
their oral or skin microbiomes. What makes microbiomes distinct from each other is the diversity of species that make up specific microbiomes. Most often the species measured is bacteria. In a typically healthy human, the skin microbiome will show increased presence of *Propionibacterium* and *Staphylococcus* compared to the mouth which will show a higher presence of *Streptococcus* and *Rothia* (4). Due to differing diversities, each microbiome functions in different ways to support the area of the body that it exists in. Another characteristic of the human microbiome is that it is unique to the individual. Every person has a different microbiome as unique to them as their fingerprint. For the purposes of this literature review, the microbiome of most interest is the gut microbiome.

The gut microbiota, a term for the population of microbes in the gut rather than just the genetic material, has been implicated in many essential functions. These essential functions include regulation and stimulation of the immune system, synthesis of vitamins, out competition of pathogens, and fermentation of food components into absorbable metabolites (5). Many of these functions are interconnected and play a role in not only gut health but overall health of the body.

Firmicutes, Bacteroidetes, and Proteobacteria are phyla of bacteria that have the largest presence in the gut (6). Firmicutes make up the largest portion of bacteria in the gut and they consist of Gram positive bacteria including the genera *Clostridium*, *Lactobacillus*, *Eubacterium*, *Faecalibacterium*, and *Roseburia*. These bacteria have a variety of functions including probiotic functions and butyrate production, which is a rich energy source for cells. Bacteroidetes are the second most abundant phyla and consist of Gram negative bacteria including the genera *Bacteroides*, *Prevotella* and *Xylanibacter*. Other phyla including Proteobacteria and Actinobacteria are less abundant and include
relevant genera such as *Escherichia* and *Desulfovibrio* and *Collinsella* and *Bifidobacterium* respectively. The diversity of bacteria in the gut varies on an individual level and can be influenced by such things as age, diet, and disease (7).

Firmicutes are Gram positive bacteria that make up the most abundant phyla in the gut microbiota. *Faecalibacterium prausnitzii* is the most abundant Firmicute found in the gut and is associated with reduction of low-grade inflammation. *F. prausnitzii* is reduced in cases of obesity; however, on a phylum level, the general population of Firmicutes is increased. The positive correlation between Firmicute abundance and obesity is likely due to the ability of Firmicutes to more completely degrade energy sources. The Firmicute phylum is the main producer of butyrate, a favorable short chain fatty acid (SCFA) produced through the fermentation of nondigestible carbohydrates. SCFA are an energy source for colon cells (colonocytes) and promote intestinal barrier integrity. Intestinal integrity is key to maintaining a healthy gut microbiota. Additionally, SCFA lowers the luminal pH, inhibiting the growth of pathogenic bacteria (8).

*Clostridium* and *Lactobacillus* are abundant genera of the Firmicute phylum found more predominantly in the mucosal lining of the gut than other genera. These phyla breakdown polyphenolic compounds, secondary metabolites found in plants that are shown to have positive health effects as well as antimicrobial activities (9).

Bacteroidetes is a phylum of Gram negative bacteria commonly found in the gut microbiota. Gram negative bacteria possess an outer membrane made up of lipopolysaccharides (LPS) and fatty acids. LPS can stimulate the dendritic cells of the immune system. Activation of dendritic cells ultimately shields the gut microbiota from unnecessary immune responses to the commensal microbes living in the gut. Similar to
Firmicutes, Bacteroidetes produce SCFA, most commonly propionate and acetate. Propionate plays a role in gluconeogenesis, the production of glucose, and acetate serves as a substrate for cholesterol (8). Common genera found in the Bacteroidetes phylum include *Bacteroides* and *Prevotella*. *Bacteroides* are proinflammatory bacteria that participate in carbohydrate and polyphenol metabolism. Some diseases such as obesity and Crohn's Disease show decreased levels of *Bacteroides*, while other diseases such as type 1 diabetes show increased levels of Bacteroides. *Prevotella* and *Bacteroides* do not exist in equal proportions. *Prevotella* maximizes energy intake from plant-based diets (10) whereas *Bacteroides* maximize energy intake in protein and fat rich diets.

The ratio between Firmicutes and Bacteroidetes in the gut is correlated with fluctuations in diet as well as disease prevalence. In cases of obesity, the abundance of Bacteroidetes is decreased while the abundance of Firmicutes is significantly increased. Heavy-fat diets alter the gut microbiota in favor of Gram-negative bacteria thus increasing production of and intestinal permeability to LPS. LPS induces an innate immune response causing low-grade inflammation, which is characteristic of many intestinal diseases including obesity. Interestingly, the Gram-negative phyla is Bacteroidetes, not Firmicutes, which, as stated previously, is the more abundant phyla in cases of obesity. *Prevotellaceae*, a family in the Bacteroidetes phyla, may be responsible for this conundrum. An increased abundance of *Prevotellaceae* is associated with obesity, contributing to the increased presence of LPS (8). Non-alcoholic fatty liver disease (NAFLD) is associated with an increased abundance of Firmicutes and a reduction in Bacteroidetes. Similar to obesity, NAFLD is strongly influenced by a high-fat diet. It was found that in mice colonized with specific species of Firmicutes compared to mice
colonized with Bacteroidetes, had increased expression of genes related to fatty acid influx and lipogenesis promoting fat accumulation in the liver (11). Findings related to the disturbance of microbial diversity in the gut related to diseases like obesity and NAFLD display the significant role diet plays on shaping microbiota diversity.

Alterations to diet can influence the abundance of microbial species in the gut. Fermented food impacts diversity through the direct consumption of probiotic species, typically Firmicutes such as *Lactobacillus* and Acinetobacter such as *Bifidobacterium*. Diet changes can therefore influence the state of diseases where gut dysbiosis is seen, including diseases beyond the gut like Alzheimer’s disease. It is also important to note that diet is not the only influential factor on gut microbial diversity. The impact of disease on the gut microbiota is two-fold. The gut microbiota has a role in the pathogenesis of disease, at the same time, disease has an impact on the gut microbiota. In future sections the relationship between the gut microbiota and neurological diseases will be discussed.

The third most abundant phyla in the gut microbiota is Proteobacteria. Proteobacteria are Gram negative and can be broken down into six classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, and Zetaproteobacteria. While Proteobacteria are less abundant than Firmicutes and Bacteroidetes in the gut, their relative abundance can still be indicative of disease. Some of the causative agents of intestinal infectious disease come from the Gammaproteobacteria class including *Escherichia, Shigella, Salmonella,* and *Yersinia*. Another intestinal pathogen, *Helicobacter*, belongs to the Epsilonproteobacteria class. Increased abundance of Proteobacteria, particularly *Enterobacteriaceae*, a family of Gammaproteobacteria, has been associated with liver damage, heart disease, obesity,
and inflammatory bowel diseases. The depletion of oxygen by colonocytes and the increase in nitrate under inflammatory conditions may produce a favorable environment for Proteobacteria, promoting their growth and overall gut dysbiosis (12). Another family of Proteobacteria, *Desulfovibrionaceae*, had an increased abundance in children with autism. However, Proteobacteria are not always bad. *Oxalibacterium formigenes*, a Betaproteobacteria, promotes the homeostasis of oxalic acid and prevents the formation of kidney stones (10). Nonetheless, an increased abundance of Proteobacteria in the gut typically reflects dysbiosis or an unstable gut microbial community structure (13). Despite its low numbers in the gut microbiota, Proteobacteria are a useful tool for understanding the role of gut microbial diversity in health and disease.

Pathogenic infections of Proteobacteria promote inflammatory responses that are implicated in diseases all over the body. Persistent *Helicobacter pylori* infections have been implicated as a causative agent in Alzheimer’s disease. A massive release of inflammatory mediators increased amyloid concentration, and entanglement of tau proteins can occur as a result of *H. pylori* infections and promote the development of Alzheimer's disease. This correlation further establishes the connection between gut microbial diversity and disease.

**C. The Gut-Brain Axis**

The gut microbiota has an impact on the physiological regulation of many parts of the body including the function and maturation of the immune system and the function, behavior, and brain development of the nervous system. The relationship between the gut microbiota and the brain is known as the gut-brain axis (14). The gut-brain axis is a bi-
directional communication system that provides microbes and their metabolites with a path to access the brain via neural, hormonal and immunological pathways (15). There are a variety of potential mechanisms the gut microbiota and the central nervous system (CNS) use to communicate with each other. Some of these mechanisms include diversity of the microbiota, the immune system, microbial metabolites, the vagus nerve, and the hypothalamus–pituitary–adrenal axis (HPA). Most of these pathways including the immune system and the vagus nerve work in a bidirectional fashion whereby the brain can influence the gut microbiota, and the gut microbiota is capable of influencing the brain. The gut microbiota can indirectly influence the limbic system and the HPA through microbial metabolites/ neurometabolites and the immune system. Likewise, the CNS indirectly influences the gut microbiota through production of neurotransmitters and hormones produced via the HPA (16).

The bidirectionality of the gut-brain axis is evidenced by the ability of the gut microbiota to modulate the brain and the ability of the brain to alter intestinal biology, bacterial composition and immune function of the gut (14). For communication between the gut microbiota and the brain to occur, passage through the blood brain barrier (BBB) and the intestinal barrier must be possible. The permeability of these barriers is influenced by the gut microbiota and the CNS. A layer of epithelial cells and a layer of mucus make up the intestinal barrier. M-cells in the epithelial layer facilitate the translocation of microbes and their metabolites into lymphatic tissue, promoting communication with the immune system. The mucus layer provides physical protection for the rest of the epithelial cells from microbes. Permeability of the intestinal barrier can be influenced by the autonomic nervous system and stress. The blood-brain barrier
separates cerebral spinal fluid in the CNS from the circulatory system. Permeability of the BBB is influenced by immune activation and SCFA produced by microbes (17).

Disruption of the gut-brain axis communication system has pathological consequences related to both the gut (such as irritable bowel syndrome) and the brain (neuroinflammatory, neurodegenerative, and behavioral disorders). Communication between the gut microbiota and the brain is very complex and still not completely understood; however, it is clear they strongly influence each other. Given this influence it is possible that modulation of the gut microbiota can improve neurological conditions, including neurodegenerative disorders like Alzheimer’s disease.

In order to understand how alterations to the gut microbiota can impact diseases like Alzheimer’s, it is important to first understand the means of communication that the gut-brain axis uses and how the nervous system and the gut microbiota are interacting with each other. As stated previously, some of the main communication mechanisms that support the gut-brain axis are the vagus nerve, the immune system, microbial metabolites, the HPA axis, and diversity of the microbiota.

One of the mechanisms that supports the interaction between the brain and gut microbiota involves the vagus nerve. The vagus nerve is key to the communication between the CNS and the gut microbiota and carries signals in both directions. Many of the effects the gut has on the brain and vice versa are dependent on the vagus nerve. For example, the ability of *Lactobacillus rhamnosus* to stimulate the transcription of γ-aminobutyric acid (GABA), a neurotransmitter, receptors is dependent on the vagus nerve (18). Furthermore, supplementation with probiotics in mice that had their vagus nerve removed does not produce the ameliorative effects that would normally occur.
Conversely, the intestines receive signals from the CNS via the vagus nerve and efferent fibers which control intestinal motility, release of neurochemicals, and the intestinal immune environment (14).

Another mechanism of communication involves the immune system. The immune system is made up of cells and organs that work together to protect the body from disease. Both the gut microbiota and the CNS communicate with each other through the immune system using neurotransmitters and microbial metabolites. Stimulation of the innate and adaptive immune responses results in the production of immunomodulating factors (cytokines, complement, major histocompatibility complex class 1 proteins) and the activation of immune cells (14). The immune system can then produce anti-inflammatory and pro-inflammatory responses that directly influence brain function and gut microbial homeostasis. Inflammatory responses that occur as a result of the gut microbiota are facilitated by the translocation of microbes and microbial metabolites across the intestinal barrier (16). A gut microbiota that is functioning properly (14) induces the maturation of the immune system and protects the intestinal wall barrier by reducing permeability (18). A damaged gut wall as a result of dysbiosis and disease increases gut permeability thus increasing translocation of bacteria into lymphoid tissue promoting inflammatory responses. Inflammatory responses then influence the central nervous system and alters its function (18). Immune cells in lymph tissue recognize pathogen associated molecular patterns (PAMPs) on microbes found in the gut microbiota resulting in immune responses that are then relayed to the brain.

The gut microbiota can influence the immune system through the production of metabolites. *Bacteroides fragilis, Bifidobacterium, Lactobacillus*, and SCFA are anti-
inflammatory, while *Streptococcus, E. coli, Enterobacteriaceae*, and segmented filamentous bacteria are pro-inflammatory. Microbes, and their metabolites, influence inflammatory responses by altering the production and function of immune cells. For example, segmented filamentous bacteria promote the development of T helper 17 cells that produce proinflammatory factors (IL-17A) whereas *B. fragilis* and SCFA promote IL-10-producing regulatory T cells that down-regulate the immune system. The colonization of the gut microbiota with specific members encourages either pro- or anti-inflammatory responses (14).

The CNS influences the immune system using neurotransmitters that are capable of activating various immune cell subsets. For example, dopamine is able to activate peripheral innate and adaptive immune cells and acetylcholine is known for its anti-inflammatory properties. Stimulation of the enteric nervous system with these neurotransmitters could promote either pro- or anti-inflammatory responses. Furthermore, serotonin and histamine are neurotransmitters that are known to influence the enteric nervous system.

Microbial metabolites can directly and indirectly influence the CNS. The gut microbiota directly influences the CNS through modulation of microglia and astrocytes. Microglia are immune macrophages present in the brain that are involved in proper neurodevelopment and immune functioning (14). Microglia play an important role in physiological brain function in areas including maintaining homeostasis, scavenging of pathogens and dying cells, brain development, synaptic pruning, and remodeling (19). The microbiota is capable of influencing the function and maturation of microglial cells. This can be displayed in germ free mice, where microglia development and function is
impaired (14). Microglia are essential for initial pathogen control under bacterial or viral infections of the brain. In the absence of a microbiota, the innate immune response is strongly diminished. Furthermore, disruption of the gut microbiota under antibiotic treatments mirrors the effects of microglia developed under germ-free conditions. This suggests the need for a consistent colonization of microbes for appropriate microglia homeostasis. Microbial metabolites are also influential in the function and development of microglia. SCFA including acetate, propionate, and butyrate have been shown to reverse microglia deformities in germ-free mice (19).

Astrocytes are glial cells (supportive/insulating cells) in the brain that serve many functions including regulation of the blood-brain barrier, ion gradient balance, neurotransmitter turnover, and blood flow and nutrient transport in the brain. Additionally, astrocytes are involved in metabolism and glycogen storage and immune functions. The gut microbiota influences astrocytes through the production of microbial metabolites that activate receptors on astrocytes. Type 1 interferon and indoxyl-3-sulfate (I3S), a metabolite of tryptophan, are examples of microbial metabolites that influence astrocytes. Type 1 interferon reduces inflammation and symptoms in experimental autoimmune encephalomyelitis (EAE), the murine model for multiple sclerosis. Similarly, supplementation with I3S in EAE improves disease symptoms, likely through reducing the expression of proinflammatory factors. The microbe potentially responsible for the regulation of astrocytes, Lactobacillus reuteri, is known to produce indole-3-aldehyde, a natural ligand for astrocyte receptors, through the metabolism of tryptophan (14).
The gut microbiota can also directly influence the CNS by producing neurotransmitters on their own or by encouraging other cells to release them (14). Examples of bacteria that produce neurotransmitters include *Lactobacillus* spp. and *Bifidobacterium* spp. (GABA), *Escherichia* spp., *Bacillus* spp. and *Saccharomyces* spp. (noradrenalin), *Candida* spp., *Streptococcus* spp., *Escherichia* spp., and *Enterococcus* spp. (serotonin), *Bacillus* spp. (dopamine), and *Lactobacillus* spp. (acetylcholine). Neurotransmitters released by the gut microbiota influence immune cells which can go on to alter brain functioning, further displaying the importance of the immune system in the interaction between the gut and the brain (16). Another way the gut microbiota influences the brain is through neurochemical modulation. This can be done by producing or changing neurotransmitters or altering neurotransmitter receptors (20).

Indirect pathways require the participation of the immune system and the HPA. The HPA axis is a neuroendocrine system that influences many organ systems in response to physical or psychological stress. The HPA response to stress encourages changes in intestinal permeability, motility, and mucus production. These alterations influence the composition of the gut microbiota. Hormones released by the HPA influence immune responses, both pro-inflammatory and anti-inflammatory. The HPA can be influenced by both the CNS and the gut microbiota. CNS modulation of the HPA promotes the release of signaling molecules that promote the release of hormones that stimulate the HPA in response to stress (14). The gut microbiota plays a role in the development of the HPA axis. This can be displayed in stress studies where germ-free mice are shown to have increased levels of stress inducing hormones compared to colonized mice. Furthermore, colonization of mice with *Bifidobacterium infantis* reverses
the stress response in the germ-free mice (16). The gut microbiota has an influence on the brain through the HPA axis. Hyperactivity of the HPA is associated with cognitive deficit. In a study analyzing the relationship between the HPA and the gut microbiota, it was found that germ-free mice had an exaggerated stress response to being restrained compared to mice that had an early developed gut microbiota. It was also found that the stress response could be reduced when probiotics were administered (20).

All of the microbial characteristics that influence these communications systems (SCFA, neurotransmitters, interleukin, etc.) can be found in bacteria commonly used in fermented foods including *Lactobacillus* and *Bifidobacterium*. The ability of these probiotic bacteria to colonize the gut and influence the gut-brain axis communication systems could prove useful in diseases where these systems are dysfunctional or play a role in disease pathology.

Diversity of the gut microbiota is another communication tool used by the gut microbiota and the CNS. The CNS can influence and be influenced by the diversity of the gut microbiota. Microbial diversity influences the CNS in many developmental stages. For example, microglial development is influenced by the diversity of the gut microbiota. The diversity of the microbiota offspring are exposed to at birth has been shown to influence the development of neurological disorders later on including autism and anxiety. The CNS indirectly alters the diversity of the gut microbiota through the promotion of a pro-inflammatory response, inducing stress responses, and development of neurological disorders (14). For example, stress potentially induces dysbiosis through the production of noradrenaline which alters bacterial gene expression (15).
Given the complex relationship between the gut microbiota and the brain and the various means of communication, disruption to any aspect of this axis can have detrimental consequences. Studies examining the development of the gut microbiota and nervous system in subjects over a lifetime displays the intricacies of the gut-brain axis. Alterations to the gut microbiota influences changes in the nervous system and vice versa, affecting both general and mental health. In early development, disruptions to the gut microbiota negatively impacts the relationship between the gut microbiota and the nervous system resulting in increased risk for neurodevelopmental disorders. Alterations to the gut-brain axis have been associated with some neurological disorders including autism spectrum disorder, Parkinson’s disease, Alzheimer’s disease, multiple sclerosis, and mood disorders. The diversity of the gut microbiota changes as people age. This could serve as a potential link to some diseases commonly experienced by elderly people, including Alzheimer’s disease. However, little conclusive evidence exists supporting the underlying mechanisms or role of the gut microbiota in the pathogenesis of these neurological diseases (18).

Considering the ability of gut microbial disruptions to influence alterations in the brain, it is within reason to expect a reversal of those brain alterations if the gut microbiota can be shifted back to its appropriate state. The use of probiotics to remedy alterations in the gut microbiota of individuals with neurological diseases has been analyzed; however, the mechanisms of action are unclear. One study found that mice with EAE treated with *Lactobacillus* strains (*L. paracasei, L. plantarum*) had suppressed disease progression and reversed disease establishment by downregulating T-cells. Interestingly, all three strains of *Lactobacillus* used had to be present in order for the
therapeutic effect to occur. Another study analyzing the effects of a probiotic consisting of *Streptococcus thermophilus, Lactobacillus reuteri, Bifidobacterium bifidum, Lactobacillus acidophilus* and *Lactobacillus casei* in mice before and after the development of EAE found that mice treated with the probiotic before EAE induction had decreased incidence of EAE and suppressed progression in mice that developed EAE. Much of the research investigating the mechanisms of probiotics on neurological diseases have focused on autism spectrum disorder (ASD). Most of the probiotics used in these studies consisted of a *Lactobacillus* species and had a variety of effects including reduced incidence of ASD and improvements in concentration, anxiety, anti-social behavior, and communication. The promising work on ASD provides a hopeful approach to investigating probiotic effects in other neurological disorders. In most of these studies, the probiotic is a collection of intentionally selected strains administered using typical medicinal techniques. Another way to introduce probiotics is through fermented food. Furthermore, fermented food has the added benefit of tasting good and producing a variety of additional compounds that are beneficial to health (18).

The complexities of the gut-brain axis relationship are not completely understood. However, changes in the gut microbiota are associated with neurological problems and vice versa. An interesting field of research is one that investigates the use of probiotics to reverse some of the symptoms related to neurological disorders. Like most of the research related to this field, the exact means by which probiotics induce these beneficial actions is not completely understood. Nonetheless, much of the influence of probiotics can be related to their ability to use the gut-brain axis communications systems to have an effect on both the brain and the gut. The use of fermented food as a potential probiotic to
influence changes in neurological disorders, such as Alzheimer’s disease, is an interesting and emerging field of research.

II. Fermented Food and the Gut Microbiota

Recent investigations into the health effects of consuming fermented food have shown associations with weight maintenance, reduced risk of cardiovascular disease, type two diabetes, and overall mortality, as well as proposed benefits related to immune pathologies and brain activities. Many of these health effects are related to the probiotic function of fermented food and its ability to produce bioactive compounds and metabolites (21).

The World Health Organization defines a probiotic as living organisms which in adequate amounts can confer health benefits to the host. Fermented food can act as a mechanism of entry for common probiotic strains (22). The main microbes present in probiotics are lactic-acid bacteria (*Lactobacilli, Lactococci* and *Bifidobacteria*) or yeasts (*Saccharomycetes*). *Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus johnsonii, Bifidobacterium* and *Saccharomyces boulardii* are the most researched strains. Probiotics have many mechanisms that work together to exert health benefits. Mechanisms of action include inhibiting the colonization of pathogenic bacteria through competition and production of antimicrobial compounds, production of nutritive compounds including vitamins and enzymes, regulating intestinal transit, supporting the intestinal barrier, and regulating adaptive and innate immune responses. As a result of these functions, fermented food containing these common probiotic strains can influence a variety of physiological functions including brain
function (18). Consumption of fermented foods including sauerkraut, kimchi, kefir, natto, yogurt, and tempeh could introduce a new, temporary community of microbes into the gut microbiota by increasing the number of microbes in the diet substantially (21).

Examples of fermented foods that have been shown to induce probiotic effects whereby they alter the microbial diversity of the gut microbiota include kefir, tempeh, natto, and kimchi. Kefir, a fermented milk made from kefir grains, has been shown to alter the gut microbiota. A large variety of microorganisms have been found in kefir including *Lactobacillus brevis, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus kefiri, Lactococcus lactis, Streptococcus thermophilus, Acetobacter lovaniensis, Acetobacter orientalis, Saccharomyces cerevisiae, Candida Kefyr, Kluyveromyces marxianus* and *Leuconostoc mesenteroides*. Investigations into the impact of kefir on the gut microbiota indicate an increase in *Lactobacillus, Lactococcus* and *Bifidobacterium* concentrations, and reductions in *Proteobacteria* and *Enterobacteriaceae* concentrations. The microbial reductions could be related to the antimicrobial activity seen in kefir. The abundance of yeast was also increased after regular kefir consumption. Tempeh is a fermented soy product and is shown to increase the relative abundance of *Bifidobacterium, Lactobacillus, Escherichia coli* and *Enterococcus* in an in vitro gut simulator model. Natto is another fermented soy product produced through fermentation of cooked yellow soybeans with *Bacillus subtilis*. Consumption of natto was shown to increase the abundance of *Bacillus* and *Bifidobacteria* in the gut microbiota and decrease *Clostridia* and *Enterobacteriaceae*.

Kimchi is a Korean food that is made from fermented vegetables. Upon initial fermentation, kimchi has a mixed variety of microbes present, however, as fermentation
continues, *Leuconostoc* is the dominant genus. Kimchi has been found to increase the abundance of *Lactobacillus* and *Leuconostoc* in the gut microbiota (3).

In addition to its probiotic function, fermented food contains microorganisms capable of producing bioactive compounds and metabolites. Enzymatic activities of microorganisms present in fermented food influence the nutritive and bioactive properties of food. A variety of fermented milks, grains, fruits, and vegetables contain microbes capable of producing bioactive compounds. A significant property of bioactive compounds is their antioxidant ability (21). Antioxidants protect against oxidative damage caused by free radicals that are by-products of physiological functions in the body. Enzymes such as superoxide dismutase, glutathione peroxidase, and catalase as well as non-enzymes including vitamin C, phenolic compounds, and carotenoids protect the body from oxidation. Some microorganisms can produce antioxidants through the degradation of food components (21).

Fermented milk products such as yogurt and kefir are made using lactic acid bacteria (LAB), *Bifidobacterium*, and yeast microorganisms that contribute to flavor, texture, and nutritional value. In addition to these functions, microorganisms present in fermented milk produce bioactive components with antioxidant, anti-hypertensive, anti-diabetic, and anti-allergy properties. Fermented milk products produce antioxidants through the release of bioactive peptides during proteolysis of milk proteins such as α-lactalbumin, β-lactoglobulin, and α-casein. The microorganism strain and the fat content of the fermented milk product influence its antioxidant capacity. Conjugated Linoleic Acid (CLA) and folate also have antioxidant properties and can be found in fermented
milk products. Fermented fruits, vegetables, and grains including kimchi, sauerkraut, and tempeh also have antioxidant properties (21).

Vitamins are another bioactive compound microorganisms can produce. Microorganisms such as LAB and *Bifidobacteria* can produce vitamins including folate (B9), riboflavin (B2), vitamin 12, and vitamin K. Some vitamins such as folate (B9) possess antioxidant capabilities. Other vitamins are equally essential for a variety of other bodily functions (23).

Many other health promoting functions can be attributed to fermented foods. Anti-hypertensive peptides can be produced through the degradation of milk proteins in fermented milk products. Anti-hypertensive peptides work by inhibiting Angiotensin-Converting Enzyme (ACE) which is critical for blood pressure regulation. Milks fermented with *Lactobacillus lactis* were found to have ACE inhibitory peptides. Fermented foods can also have anti-inflammatory properties (23). In one study, cheese cultured with species known to be anti-inflammatory was capable of protecting against colitis in a mouse model (21). *Lactobacillus* strains present in fermented foods enhance intestinal barrier integrity thus maintaining immune tolerance by preventing bacterial translocation across the intestinal barrier. This reduces the risk of developing inflammatory diseases in the gut. Finally, fermented food has anti-allergy properties (22). The initial digestion of food products by microbes present in fermented dairy products contributes to the improved tolerance of these dairy products in people with lactose intolerance (21). Consumption of yogurt improves lactose tolerance in lactose intolerant people due to the production of lactase by LAB (23).
Fermented food can have neurological impacts on the brain directly through the production of neuroactive compounds and indirectly through modulation of the gut microbiota diversity. Microbes in fermented food that colonize the gut can communicate through the gut-brain axis using neuroendocrine and neuroactive compounds such as serotonin, GABA, histamine, noradrenaline, and adrenaline ultimately enabling fermented food to have a neurological impact. For example, *Lactobacilli*, a common bacterium of fermented food, are capable of converting glutamate into GABA, an inhibitory neurotransmitter known to play a role in neurological diseases including AD (22).

Probiotics can modulate the intestinal immune system by producing metabolites related to the growth and function of intestinal immune and epithelial cells. *Lactobacillus reuteri* is a Gram positive bacteria commonly found in the gut microbiota that is capable of regulating the intestinal immune system. Possible mechanisms for regulating the immune system used by *L. reuteri* include production of anti-inflammatory cytokines, inhibition of pro-inflammatory cytokines, and reducing recruitment of immune cells. *L. reuteri* is a non-starter lactic acid bacteria (NSLAB) commonly used in fermented milk products. In one study, mice with EAE treated with probiotics *L. paracasei* and *L. plantarum* displayed a reduction in EAE symptoms. *L. paracasei* and *L. plantarum* are commonly found in fermented fruit and vegetable products (22).

Research into the beneficial health effects fermented food has on the nervous system, including neuroprotection and cognitive enhancement, has been conducted on a variety of fermented milks, grains, and vegetables. Most of the health effects related to fermented foods are a result of chemical changes that make certain beneficial compounds
more bioavailable. Examples of fermented milk products that have neuroprotective effects include Camembert cheese extract, fermented soy milk, and Calpis sour milk. Camembert cheese extract suppresses neuronal death brought on by excessive microglial activation as well as reduces the accumulation of Aβ plaques in the brain of people with Alzheimer’s disease. Fermented soy products have a neuroprotective effect against reactive oxygen species and Calpis sour milk has been shown to improve memory and reduce stress. Fermented grains and vegetables are also capable of improving memory and cognitive enhancement. Kimchi has also been associated with anti-carcinogenic, antibacterial, and anti-oxidative properties (20).

III. The Gut Microbiota & Alzheimer’s disease

Alzheimer’s disease is the most common form of dementia in old people. The exact cause of Alzheimer’s disease (AD) is unknown; however, associations with diet, exercise, cognition and aging, infections, chronic inflammation, and many other factors have been linked with AD (24). Additionally, a number of genes have been connected to AD including some related to microglia. The pathogenesis of AD is also not entirely clear; however, the accumulation of amyloid peptides and tau proteins in the brain and their resulting inflammatory response has been the primary hypothesis (19). Amyloid beta (Aβ) plaque formation and tau protein accumulation trigger neuroinflammation resulting in synapse loss and neuronal death (25). Amyloid is a general term for an insoluble, lipoprotein rich deposit exhibiting β-pleated sheet structures (26). APP, a transmembrane protein involved in various biological processes, produces Aβ upon cleavage. Secretases break down APP forming neurotoxic Aβ peptides that aggregate and
form plaques. Pieces of the plaque break off and are capable of seeding in different areas of the brain. What triggers amyloid plaque formation remains unknown. Tau is a soluble protein that provides stability to axonal microtubules. Aggregation of these proteins act as a toxic stimulus for neurodegeneration in AD.

There are many possible triggers for AD development, of particular interest is the role the gut microbiota plays in the triggering of AD (25). As people age the diversity of their gut microbiota changes with decreases in Bifidobacterium and SCFA production both of which are anti-inflammatory. These disruptions likely account for chronic inflammatory reactions in elderly people (26). Chronic inflammatory infections have been linked to the onset of AD as have gut inflammation, dysbiosis, and increased intestinal permeability (24, 25). Plasma levels of β-amyloid peptides are increased in AD patients infected with H. pylori or Borrelia burgdorferi and Chlamydia pneumoniae (24).

Changes in gut microbial diversity are characteristic of AD and may account for the onset of disease. Broad changes in gut diversity include a decreased abundance of Firmicutes and Actinobacteria, and an increased abundance of Bacteroidetes and Proteobacteria in people with AD. More specifically, the families within the Firmicutes phylum that are reduced include Ruminococcaceae, Turicibacteraceae, Peptostreptococcaceae, Clostridiaceae, and Mogibacteriaceae. Within the Bacteroidetes phylum, an increase in the families, Bacteroidaceae and Rikenellaceae is seen. Of particular interest is the decrease in Acinetobacteria marked most notably by a decrease in the family Bifidobacteriaceae. In a more general sense, AD patients experience an increase in pro-inflammatory bacteria (Bacteroidetes and Proteobacteria such as Escherichia and Shigella), and a decrease in anti-inflammatory bacteria (Firmicutes,
Bifidobacterium, and Eubacterium rectale). Several studies investigating the altered gut microbiota of AD patients have been conducted with varying degrees of results. More research needs to be done in order to form a stronger correlation between gut health and AD (27).

Consequences of gut dysbiosis are related to the pathogenesis of AD. Dysbiosis increases the concentration of pro-inflammatory molecules including cytokines and bacterial components/metabolites, the destruction of the blood brain barrier and intestinal barrier integrity, and the dysfunction of microglia.

Cytokines are produced by immune cells in response to antigens resulting in a pro-inflammatory response that facilitates the removal of the antigen. Many factors contribute to the increased concentration of cytokines including presence of LPS, overactive microglia, and a high fat diet. Pro-inflammatory metabolites produced by gut bacteria exacerbate AD by intensifying neuroinflammation and increasing the aggregation of amyloid and tau proteins. Endotoxins produced by certain species of bacteria in the gut are also associated with neuroinflammation and amyloid fibril formation. In addition to their ability to promote the aggregation and formation of amyloids, certain gut bacterial species can form amyloids themselves. Some Enterobacter species and fungal species can produce amyloid peptides or amyloid fibers that are able to form new foundations for amyloid aggregation in the brain (24). An example of an amyloid found in the gut is curli produced by Escherichia coli. Amyloids help bacteria bind together to form biofilms so they can resist physical and immune destruction. While the amyloids produced in the gut are different than those produced in the brain, immune
activation to gut amyloids could have a priming effect resulting in increased immune responses to amyloids in the brain (25).

Some metabolites produced by the gut microbiota, such as SCFA, can have beneficial neurological effects. The effect of metabolites such as valerian, isovaleric, isobutyric, butyric, propionic, acetic and formic acids on AD has been analyzed and found to influence AD development through interference with astrocyte and microglia activation. This interference reduces inflammation and aggregating tau protein and amyloid. Propionic, butyric and valeric acids inhibit oligomerization of some amyloid peptides (24). Additionally, butyrate can increase motility, attenuate neural deficits, restore the blood-brain-barrier, and improve memory and learning. Propionate can decrease motility and increase secretions. Under the dysbiotic conditions experienced in AD, the abundance of microbes that produce these beneficial metabolites is reduced, intensifying AD (26).

Components of the bacteria themselves can exacerbate AD symptoms by facilitating neuroinflammation. Plasma and hippocampal concentrations of LPS are higher in AD patients than in people without AD (25). Increased abundance of Gram-negative *Bacteroides* in people with AD results in increased translocation of LPS from the gut to systemic circulation. The presence of LPS in systemic circulation contributes to/ intensifies AD pathology through inflammatory brain degeneration. In addition to neuroinflammation (27), LPS promotes accumulation of Aβ peptides (24). As people age, the barriers in the brain and gut become more permeable increasing systemic exposure to bacterial components and metabolites like LPS and amyloid (26). The decreased
abundance of *Bifidobacterium* in AD further promotes the increased abundance of LPS since *Bifidobacterium* has been shown to decrease LPS levels in the intestines (27).

Disruptions to both the blood-brain-barrier (BBB) and the intestinal mucosal barrier contribute to the pathogenesis of AD. The BBB controls molecule transportation in and out of the brain. Inflammation-related BBB decay could result from alterations to the gut microbiota. The gut epithelial barrier facilitates contact between the gut microbiota and the submucosal lymphoid tissue. Damage to the intestinal barrier makes it easier for pro-inflammatory bacterial components/metabolites to enter systemic circulation which could ultimately result in inflammation in the brain (26). For example, bacterial amyloids from the gut enter systemic circulation when gut permeability is increased. The diversity of the gut microbiota can influence the permeability of both the intestinal and blood-brain barriers (24). Dysregulation of the intestinal microflora results in increased permeability of both the intestinal barrier and the BBB (26). *Lactobacillus plantarum*, *E. coli*, and *Bifidobacterium infantis* enhance the intestinal barrier by increasing expression of tight junctions thus decreasing permeability. However, pathogenic microbes including *E. coli* strains, *Salmonella*, *Shigella*, *H. pylori*, *Vibrio*, or *Clostridium* can alter tight junctions increasing permeability. The endotoxin produced by *Bacteroides* also disrupts tight junctions (25). An increased abundance of *Bacteroides* and decreased abundance of *Bifidobacterium* in AD contributes to the propensity for translocation of pro-inflammatory bacterial components and metabolites. Supplementation with *Bifidobacterium* has been shown to improve gut mucosal barrier properties in mice (27).
Finally, dysbiosis influences the function and development of microglia, the immune phagocytic cells in the brain. Amyloid deposition in the brain is not solely responsible for the development of AD. As more and more research is conducted, it is becoming more clear that the presence of the plaques themselves do not cause AD, but the inflammation brought on by the plaques. Under normal circumstances, microglia clear amyloid deposits resulting in a neuroinflammatory response. As people age, immune responses become dysfunctional and microglia have a decreased ability to clear plaques or pathogens leading to detrimental brain effects. Constant microglia activation leads to a chronic immune response. Furthermore, constant microglia activation leads to constant astrocyte activation both contributing to neuroinflammation and blood-brain-barrier dysfunction (26). Excessive activation of microglia and increased neuroinflammation from pro-inflammatory cytokines and reactive oxidative stressors lead to neuronal and glial cell death and dysfunction (25). Dysfunctional microglia have a reduced ability to phagocytize misfolded tau proteins and amyloids (24). In the early stages of AD, microglia activation in response to low levels of Aβ promotes phagocytosis and amyloid clearance. As the disease progresses and barriers break down, microglia become less effective at removing tau protein and amyloids resulting in their accumulation and accompanying neurodegeneration (25).

Alzheimer’s disease is shrouded in mystery not only in its cause, pathogenesis, and triggers, but in its treatment as well. No cure exists for AD. Given the ties to intestinal health, recent investigations into gut microbial modifications have gained interest in reducing the effects of AD symptoms including memory loss. Studies analyzing the link between diet and AD have found a reduced risk of developing AD in
diets that consisted of vegetables, fruits, cereals, and dairy products (24). Diet is one of the most effective ways to modify the gut microbiota; therefore, food-based therapies could influence the composition of the gut microbiota ameliorating AD (25). Fermented food offers a unique diet alteration capable of not only influencing the diversity of the microbiota, but producing neuroactive compounds that will also impact the progression and development of AD.

IV. AD: Oxidation, GABAergic pathway, and the HPA

Fermented food can potentially slow the progression of Alzheimer’s disease through its probiotic effect on the gut microbiota. The ability of fermented food to alter the diversity of the gut microbiota serves as a point of action in reducing AD symptoms. The influence of gut dysbiosis on AD disease development can be reduced if the dysbiosis experienced by the gut can be restored to its healthy state. Multiple fermented foods have proven to have probiotic effects by influencing the diversity of the gut microbiota to include microorganisms that constitute the food. Natto, kefir, tempeh, and kimchi increase the concentration of microbes such as *Bifidobacterium* (Acinetobacter), *Lactobacillus*, *Lactococcus*, and *Leuconostoc* (Firmicutes) in the gut microbiota. Increased abundance of these genera in the gut microbiota directly counters the existing community of microorganisms present in the gut of people with AD. Firmicutes and Acinetobacter are anti-inflammatory phyla of bacteria that are decreased in AD. Given the influence of inflammation of the progression and development of AD, the ability of fermented food to increase the presence of anti-inflammatory bacteria in the gut could reduce the inflammation-related pathologies of AD.
Consuming fermented food products has the added benefit of introducing the body to a variety of health-promoting metabolites that cannot be found in synthetic probiotic pills. In addition to gut microbial dysbiosis, oxidative stress and a dysfunctional \( \gamma \)-aminobutyric acid (GABA) system are also present in individuals with AD. Components of fermented food including antioxidants, polyphenols, and GABA can influence AD by reducing the detrimental effects of oxidative stress, A\( \beta \) accumulation, and reduced GABA concentrations (28, 29, 30). \textit{L. plantarum} has strong antioxidant activities and preserves polyphenolic compounds in fermented foods. \textit{Bifidobacterium} and LAB present in fermented soymilk enhance antioxidant activities. Fermented fiber-rich foods such as soy germ, wheat germ, rice bran, or breads are known to increase the availability of GABA. Additionally, GABA derived from fermented food has been shown to have improved absorption compared to synthetically prepared GABA. Increased GABA concentrations in people with AD could improve their condition (31). In order for GABA produced from fermented foods to influence the CNS, it must stimulate the vagus nerve and travel up (18).

The HPA axis is another area of the body that experiences dysfunction as a result of AD. The hippocampus is one of the major sites of neuronal loss in AD. Additionally, hyperactivity in the HPA is associated with AD. Given the influence of the gut microbiota on the development and maintenance in the HPA, administration of probiotic fermented food could potentially improve the damage done to the HPA. However, since the damage done is not related to gut dysbiosis it is not likely to reverse damage (32).
CONCLUSION

There is a lot that remains unknown about Alzheimer’s disease. Many physiological dysfunctions can be seen throughout the body in people with AD. In most cases is it unclear whether these dysfunctions are a result of AD or causing AD. The main theory on the pathogenesis of AD is amyloid β plaque formation, tau accumulation, inflammation, and the resulting loss of neurons that ultimately leads to dementia. Other physiological dysfunctions related to AD include impaired GABA pathways, increased oxidation, damage to the HPA axis and gut dysbiosis. Altered diversity of the gut microbiota has increasingly been shown to influence a variety of diseases. The influence of AD on the gut microbiota results in altered abundance of microbes. There is a relative increase in abundance of Bacteroidetes and a decrease in Firmicutes and Actinobacteria. Species of Firmicutes including lactic acid bacteria (*Lactobacillus, Lactococcus, Streptococcus*, and *Leuconostoc*) and Actinobacteria including *Bifidobacteria* are commonly used in fermented foods. Fermented foods have health benefits that could prove helpful in managing AD through the manipulation of the gut-brain axis. The gut-brain axis allows microbes and their metabolites present in fermented food to communicate between the gut and the brain through the vagus nerve, HPA axis, and the immune system. The probiotic abilities of fermented foods including natto, kimchi, kefir, and tempeh could reverse the dysbiosis in AD. Metabolites produced by the microbes present in fermented food could additionally reduce oxidative damage, GABA reductions, Aβ plaque formation, tau protein accumulation, and neuroinflammation all of which contribute in some way to AD.
REFERENCES


