# Effects of a 12-Week Dairy-Based Probiotic Dietary Intervention on Cognitive Outcomes in Older Adults

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## Authorization to Submit Thesis

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## Abstract

There is increasing evidence of an association between the gut microbiome and brain behavior, which has come to be known as the gut-brain axis (Gareau, 2016; Beilharz, Kaakoush, Maniam, & Morris, 2017). The gut microbiome may be a key determinant of cognitive function, encompassing processes such as memory, attention, language, problem-solving, planning, and perception (Okon-Singer, et al., 2015). Dysbiosis and alterations of the gut microbiome may contribute to the development of diseases in humans and is evident in those with neurodegenerative diseases such as Alzheimer's disease (AD) (Jiang, et al., 2017). Consistent probiotic consumption through fermented food sources may alter the gut microbiome, potentially improving cognitive outcomes and reducing cognitive decline.

This pilot study aimed to determine the effect of dairy-based probiotic consumption on cognitive outcomes among 47 older adults (ages 50-98 years old) over a twelve-week period. Participants were randomized to consume one six-ounce yogurt containing probiotics each day (treatment, n=24), or to avoid yogurt and other fermented food over the course of the study (control, n=23). Tests to assess cognitive outcomes were administered at baseline and at the end of the 12-week intervention. Analysis of variance indicated no clear evidence that daily dairy-based probiotic consumption over 12-weeks influenced cognitive outcomes in older adults.

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## List of Abbreviations

| | |
|---|---|
| AD | Alzheimer's Disease |
| GI | Gastrointestinal |
| FDA | Food and Drug Administration |
| CFU | Colony-Forming Unit |
| ENS | Enteric Nervous System |
| CNS | Central Nervous System |
| GABA | Gamma-Amino Butyric Acid |
| BBB | Blood-Brain Barrier |
| NIH | National Institute of Health |
| SAS | Statistical Analysis Software |
| ANOVA | Analysis of Variance |

# Chapter One: Literature Review

The microbiome of the human gut is incredibly diverse and is subject to fluctuations due to factors such as change in diet or disease state (Abraham, et al., 2016). The typical Western diet has been shown to have negative effects on brain behavior and cognitive function, with specific impacts on the hippocampus, a brain structure important for learning and memory (Beilharz et al., 2017). This area of the brain is particularly sensitive to changes in diet, showing memory deficits after one week of a change in diet (Beilharz et al., 2017).

Probiotics are increasingly seen in a healthy diet and may positively alter the gut microbiome in humans. A major health benefit of probiotic consumption is the lowering of the pH in the gastrointestinal (GI) tract, which helps change the environment to one in which pathogenic organisms cannot survive (Williams, 2010). Another major health benefit of probiotics is enhancement of the immune response that the gut flora helps to regulate (Williams, 2010; Nagpal et al., 2012). Probiotics in yogurt have been considered a health-promoting food since the early 1900s (Lourens-Hattingh & Viljoen, 2001). Modern yogurt has been formulated to also include strains of *Lactobacillus acidophilus* and *Bifidobacterium* (Lourens-Hattingh & Viljoen, 2001). This probiotic-rich yogurt may have anti-inflammatory and antipathogenic effects (Adolfsson, Meydani, & Russell, 2004).

## Probiotics

By definition, probiotics are live nonpathogenic microorganisms administered in amounts that have positive effects on the health of the individual consuming them (Granato, Branco, Nazzaro, Cruz, & Faria, 2010; Williams, 2010). Many probiotics are recognized as dietary supplements and are therefore not required to meet the same standards that pharmacological medications are mandated to have by the Food and Drug Administration (FDA) (Williams, 2010). Probiotics can be identified and categorized by their genus, species, and strain level (Mizock, 2015). Probiotics are most commonly seen as Saccharomyces yeast or lactic acid bacteria, such as the species Bifidobacterium and Lactobacillus (Williams, 2010). Dietary supplements containing probiotics can offer a range from one to a few billion different species of bacteria or yeast strains.

A major health benefit of probiotic use is the anti-inflammatory effects that consistent probiotic consumption may exhibit and lowering of the GI tract pH (Plaza-Díaz, Ruiz-Ojeda, Vilchez-Padial, & Gil, 2017). The bacteria in probiotics produce lactic, acetic, and propionic

acids that help change the living condition to one in which pathogenic organisms cannot survive (Williams, 2010). The other major health benefit of probiotics is enhancement of the immune response that the gut flora helps to regulate (Nagpal et al., 2012; Williams, 2010). The boosting of the immune system develops by increasing the phagocytic activity of lymphocytes, macrophages, and various immunoglobulins (Williams, 2010). Specific strains of probiotic bacteria also have the ability to produce various substances that are harmful to invading pathogenic organisms (Williams, 2010). By recolonizing the gut with new and healthy bacteria, probiotics serve to correct what is known as dysbiosis, or microbial imbalance, which is the ultimate contributor to diseases that originate from the GI tract (Mizock, 2015). Probiotics are commonly found in supplement form, but they are also naturally occurring in a limited assortment of foods.

### *Probiotic Foods and Supplements*

Probiotics that are naturally occurring are found in what are known as "functional foods", meaning they have a potentially positive effect on a person's health. Dairy products such as yogurt, kefir, and cultured drinks that have probiotics added to them during the fermentation process represent the majority of probiotic functional foods (Granato et al., 2010; Nagpal et al., 2012). Because most of these functional foods contain dairy, they are not an appropriate source for individuals that follow a vegan diet or are lactose intolerant. Other emerging categories of functional foods include various fermented foods, soy products, fruit and vegetable juices, cheeses and ice creams, cereals, and infant formulas (Granato et al., 2010; Nagpal et al., 2012). To maximize the health benefits that probiotics provide, daily consumption of $10^7$ to $10^9$ colony-forming units (CFU) of probiotic bacteria is recommended (Nagpal et al., 2012). Based on clinical trials, this was the amount found to show the highest level of health benefits such as regulation of gut microbiome, enhancing immune system function, increasing bioavailability of nutrients, and reducing risk of certain diseases (Nagpal et al., 2012).

### *Common Strains*

Probiotics may contain billions of different species and strains of live bacteria or yeast. The billions of strains used in probiotics are mostly found within the three most common species known as *Lactobacillus* (*L.*) and *Bifidobacterium* (*B.*) (both lactic acid bacteria), and *Saccharomyces*, which is the only species of yeast used in probiotics (Mizock,

2015). Of the many species of *Lactobacillus*, the most commonly used strains include *L. acidophilus, L. acidophilus DDS-1, L. bulgaricus, L. rhamnosus GG, L. plantarum, L. reuteri, L. salivarius, L. casei, L. johnsonii, and L. gasseri* (Mizock, 2015). *Lactobacillus* strains are often found in the gut of healthy individuals, vaginal secretions of women, naturally occurring in food, and added to probiotic supplements. The *Bifidobacterium* strains commonly used include *B. bifidum, B. lactis, B. longum, B. breve, B. infantis, B. thermophilum, and B. pseudolongum* (Mizock, 2015). *Bifidobacterium* strains are derived from the gut flora and can also be found in the oral cavity and vagina. The only strain of the *Saccharomyces* species of yeast found in probiotics is *Saccharomyces boulardii*, which is a similar strain to baker's yeast (Mizock, 2015). Yogurt is a common probiotic food, and the specific probiotic strains that are commonly present in yogurt include *L. acidophilus, L. casei, and B. bifidum* (Nagpal et al., 2012). It is preferable to include strains of bacteria that are of human origin in probiotic supplements or foods because it will aid in optimal colonization of the new bacteria introduced to the gut among the pre-existing bacteria (Nagpal et al., 2012).

## Cognitive Function

Cognitive function may be broadly described as brain behavior that takes place throughout the lifecycle, including both short-term and long-term learning (Gareau, 2016). More specifically, cognition encompasses processes such as memory, attention, language, problem-solving and planning (Okon-Singer et al., 2015). Not only is cognitive function related strongly to the central nervous system for creating and storing memories, but it may also be related the immune system and intestinal microbiome (Gareau, 2016).

Different regions of the brain have a variety of functions that contribute to cognition. One of the factors that may affect cognition is diet (Beilharz et al., 2017). The hippocampus is a key region involved in learning and memory, and is particularly susceptible to negative changes in memory within one week of a change in diet (Beilharz et al., 2017). Age can also impact the structures of the brain that affect cognition. Once an individual has reached 20 years of age, episodic memory can begin to decline, with a quicker decline in later years (Goudarzvand, Rasouli Koohi, Khodaii, & Moghadam, 2016). More dramatically, after the age of 50, working memory and other cognitive functions including processing speed, spatial manipulation, and inhibitory function can begin to decline (Goudarzvand et al., 2016).

### *Causes of Cognitive Decline*

Besides aging, many factors can contribute to cognitive dysfunction, thus making it a complicated topic. While genetics and age are definite determinants of cognitive function, other lifestyle factors such as diet and exercise play a role. Cognitive dysfunction has been related to many diseases, some of which are intestinal in nature and many have been found to have a role in dysbiosis and disease pathogenesis (Kim et al., 2016). Oxidative stress induces neuronal cell death, contributing to cognitive dysfunction (Kim et al., 2016). Neuronal cell death, as a result of oxidative stress, may also be a contributor to brain pathogenesis (Kim et al., 2016). More specifically, brain functions relating to memory may be sensitive to oxidative stress, due to the relation of increased neuronal cell death. This is believed to be primarily related to the high demand of oxygen for the brain structures that support memory, such as the hippocampus (Kim et al., 2016).

### *Cognitive Function and Aging*

As mentioned previously, age-related cognitive decline is most common in individuals ages 50 and over (Goudarzvand et al., 2016). Unfortunately, a decrease in cognition in the elderly is correlated with a decrease of independence. Because of this, cognitive function is a major determinant of quality of life (Chung et al., 2014). Age-related cognitive decline is shown through an array of cognitive domains, including processing speed, attention, episodic memory, spatial ability and executive function (Camfield, Owen, Scholey, Pipingas, & Stough, 2011). As one ages, the risk for decline in any of these domains increases, which may result in a lower quality life.

## Probiotics and Cognition

The microbiome of the human body is incredibly diverse and is subject to extreme fluctuation due to factors such as diet or disease (Abraham, Dora ; Radak, Zsolt ; Feher, 2016). The dynamic nature of the internal microbiome makes for a wide range of potential health impacts. The study of the connection between the microbiome of the digestive tract, cognitive faculties, and brain function, is still in its early stages. In recent years, however, a growing body of research has been conducted that indicates a strong relationship between these realms of physiology. Currently much of the research has produced results that are largely correlative, and so the subject needs further exploration.

It is understood that there is a constant bidirectional communication between the brain and all the organs/systems of the body; this is facilitated by the parasympathetic nervous system (Galland, 2014; Hasler, 2018). There are a few different observable mechanics that illustrate how the organs of the digestive tract communicates with the central nervous system: this is known as the "Gut Brain Axis" (Hasler, 2018). Research into the Gut Brain Axis has shown that the microbiome of the digestive tract can influence these communications in the following ways: (1) Gut bacteria can stimulate afferent neurons in the digestive tract, which send signals directly to the brain via the vagus nerve (Galland, 2014). (2) Gut microbes produce hormones and neurotransmitters that are identical to those produced in humans (Galland, 2014). Fluctuations in the levels of these compounds can impact a multitude of bodily functions (Galland, 2014). (3) Bacteria can produce toxic compounds such as lactic acid and ammonia, which can have detrimental effects on neuronal tissues (Galland, 2014). (4) Microbial populations within the gut can reduce gut permeability, preventing translocation of gut bacteria into lymphoid tissue. This would ultimately activate the vagal nerve and central nervous system, altering the nervous system function. (Galland, 2014). These mechanisms and processes represent potential explanations for the correlations between the digestive microbiome and cognition.

### *Memory and Learning*

There are two overarching forms of memory: short-term and long-term. There is evidence to suggest that daily probiotic administration can improve memory dysfunction, for both long- and short-term memory, as a diverse microbiome is required for new memory formation (Gareau et al., 2011).

Each type of memory is also associated with different regions of the brain (Queensland Brain Institute - University of Queensland, 2018.). Learning is connected to neurogenesis in the adult hippocampus, which is vital for the formation of new memories (Ogbonnaya et al., 2015). Research examining the effects of probiotics and memory demonstrates that damage to hippocampal synaptic plasticity may be reversible with the administration of probiotics (Davari, Talaei, Alaei, & salami, 2013).

Memory and learning are closely related: learning requires the ability to master a task, maintain the task in short-term memory, and remember how to perform the task after long delays (Marin-Burgin & Schinder, 2012). Thus, the process of learning requires memory

acquisition (Marín-Burgin & Schinder, 2012). Much of the current literature looking at probiotic supplementation and the impact on memory and learning involves rodents, and typical methods of assessment include novel object recognition (to test spatial memory), radial arm water maze (to test spatial working and reference memory), hole-board apparatus (to test spatial discrimination), and the Morris water maze test (to test spatial memory) (Brynskikh, Warren, Zhu, & Kipnis, 2008; Hoffman et al., 2017; Li, Dowd, Scurlock, Acosta-Martinez, & Lyte, 2009). In animal studies, rats have been found to improve spatial learning, as evidenced by performance improvement in each of the above maze task settings, after 8 weeks of probiotic administration (Davari et al., 2013). Though these tests are not used to measure learning and memory changes in humans, their success among rodents lays a solid foundation for justifying further probiotic research in humans. (Davari et al., 2013).

### *Gastrointestinal Tract, Probiotics, and Aging*

The GI tract communicates endogenous changes to the brain via the vagus nerve; approximately 500 million neurons exist in the stomach alone, making up the enteric nervous system (ENS) (Li et al., 2009). The stomach is home to $10^{12\text{-}13}$ microorganisms which are in close proximity to ENS nerves, creating the microbiome-gut-brain axis (Davari et al., 2013; Li et al., 2009). Previous probiotic studies have shown that changes in gut microbiota affect hormone and neurotransmitter release (Tse, 2017a). There is a commonality of neurotransmitters in the brain and in the gut, creating a connection between CNS diseases and manifestations in the gut (Tse, 2017). It is thus possible that the composition of the gut microbiome may play some role in nervous system function.

Diet can have a significant effect on reference memory due to its ability to influence GI bacterial flora (Li et al., 2009). A "microbiome restoration diet," which focuses on heavily vegetable and protein consumption and minimal dairy, alcohol, grains, and refined sugar, has been shown to improve cognitive function, especially in regard to memory (Lawrence & Hyde, 2017). There seems to be a clear connection between the gut microbiome and memory, though the body of research on the topic currently is minimal (Lawrence & Hyde, 2017; Tse, 2017).

There is evidence to support the theory that aging decreases recognition memory and learning ability (Hoffman et al., 2017). As people age, blood flow to the frontal cortex declines, initially prompting declines in verbal fluency and executive function (Hoffman et

al., 2017). Thereafter, the parietal cortex and medial temporal area are disturbed, affecting visuomotor skills and the ability to make new long-term memories (American Psychological Association, 2006). Understanding how probiotic consumption can reverse or prevent this disturbance will be monumental in slowing down the cognitive aging process.

### *Adaptive Immunity and Memory/Learning*

The immune response provides defense against environmental or biological threats, and it has been shown that T cells, a type of immunity cell, are able to cross the blood-brain barrier (BBB) to protect against loss of neurons in the brain and enhance neurogenesis (Kipnis, Cohen, Cardon, Ziv, & Schwartz, 2004). Rodent studies have shown that strong adaptive immunity supports learning behavior (Brynskikh et al., 2008). Neurogenesis is greatly impaired in mice with immunodeficiency disorders and those deprived of T cells and lymphocytes (Marin & Kipnis, 2013). This information suggests that interaction between the immune and nervous systems contribute to normal brain function.

Evidence exists to support the theory that probiotics have immunomodulatory effects (Isolauri, Sütas, Kankaanpää, Arvilommi, & Salminen, 2001; Kober & Bowe, 2015; Yan & Polk, 2011). The surface of the stomach is protected by a "local adaptive immune system," consisting of the largest mass of lymphoid tissue in the body (Isolauri et al., 2001). Introducing probiotics into the diet has been shown to influence immune cells in the stomach (Isolauri et al., 2001). Thus, if T cells in the gut are supported by probiotics, and eventually cross the BBB to affect neurogenesis, it is possible that probiotic consumption may play an important role in memory and learning.

## Probiotics and Neurological Disease

Dysbiosis or abnormal gut microbiome may lead to diseases in humans and is evident in those with neurogenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (Jiang, et al., 2017; Sampson, et al., 2016). Patients with AD or Parkinson's are often seen with gastrointestinal comorbidities, inflammation throughout the body, and an altered gut microbiome (Jiang, et al., 2017; Sampson, et al., 2016). For this reason, it may be possible that by managing or manipulating the gut microbiome, symptoms of neurodegenerative disease may be alleviated or decreased.

## *Alzheimer's Disease*

AD is a common neurodegenerative disorder that is associated with impaired cognition (Hu, Wang, & Jin, 2016). Despite extensive research over many decades, an effective treatment to delay the onset and progression of AD has not been found (Selkoe, 2012). While the disease is not fully understood, there are a few factors about AD that are known: Those with AD typically have an altered gut microbiome, inflammation in the brain, an accumulation of amyloid-beta peptides and neurofibrillary tangles in the brain (Alkasir, Li, Li, Jin, & Zhu, 2017; Petersen et al., 2019). Previously, aging, family history, and susceptibility genes were considered to be the most important factors related to AD (Hu et al., 2016). However, more recently it has been theorized that environmental factors are actually more important than genetic factors in regards to AD (Hu et al., 2016).

It is possible that AD may have an origin in the gut, with a close relation to the imbalance of gut microbiota (Hu et al., 2016). It has been suggested that human symbiotic microbes are important environmental factors that affect the host's health and 95% of those microbes are located in the gut (Hooper & Gordon, 2001). Gut microbiota can influence host brain function, including cognition, and behavior through the microbiota-gut-brain axis (Hu et al., 2016). The disruption of the balance of gut microbiota by intestinal environment changes are directly related to leaky gut (Hu et al., 2016). Leaky gut can lead to leaky brain (increased permeability of the blood-brain barrier) which leads to a decreased ability of the brain to protect itself from toxic substances (Hu et al., 2016). Restructuring the composition of the gut microbiota has been shown to decrease permeability of the blood-brain barrier, also improving leaky gut (Hu et al., 2016). Since probiotics can alter gut microbiota, as well as gut physiology and the host's cognitive behavior, this may increase or decrease risk of AD (Hu et al., 2016). Prebiotics and probiotics can improve host cognition and have been shown to exert effective memory improvements in AD-like animals, though the mechanism is still unknown (Chen et al., 2017). Substances formed during gut microbial metabolism can also effect neurochemical changes of the host and may also increase or decrease risk of AD (Hu et al., 2016). It is possible that a potential treatment for AD in the future may involve modification and control gut microbiota through diet (Hu et al., 2016).

## Conclusion

For a probiotic supplement to contribute to health, it must contain species that are indigenous to the human gut, such as *Bifidobacterium* and *Lactobacillus* (Nagpal et al., 2012). These cultures within the human gut can affect cognition and emotional well-being through the gut-brain axis, although it is unknown if supplementation through functional foods can increase these benefits (Davari, Talaei, Alaei, & salami, 2013b; Hoffman et al., 2017b; Westfall et al., 2017). This topic is important because of the potential for use of probiotics as either a preventative method or method to decrease symptoms of neurodegenerative diseases, by way of the gut-brain axis. While previous studies show promising results, there is more research needing to be done on this topic.

# Chapter Two: Research Purpose/Overview

## Purpose

The purpose of this study was to determine the effect of daily consumption of dairy-based probiotics on cognitive outcomes in older adults over a 12-week period.

## Research Hypothesis

The present study sought to test the hypothesis that: daily consumption of six ounces of yogurt containing live probiotics over the course of twelve weeks will improve cognitive outcomes in older adults.

## Limitations

The limitations of the present study included the following:

1. Although the empty yogurt containers and consumption logs were collected weekly, daily consumption of yogurt cannot be completely confirmed.
2. A small sample size may limit generalizability to a larger population.
3. Diet of the control group cannot be completely confirmed. While participants were asked not to consume fermented foods that may contain probiotics, there is no way to accurately measure how well participants adhered to this.

# Chapter Three: Research Design & Methodology

## Study Overview

This pilot study assessed daily dairy-based probiotic consumption and its effect on cognitive outcomes in older adults (≥50 years of age) over a twelve-week period. This was a randomized study, including one treatment group and one control group. This study was part of a larger pilot study, which overall looked at the effect of probiotics on cognitive function, emotional wellbeing, and inflammatory biomarkers. Baseline and post-tests included cognitive test outcomes, emotional well-being outcomes, and inflammatory biomarkers obtained by a laboratory blood draw. For the purpose of this thesis, only the cognitive test outcomes and procedures will be discussed. Participants in the treatment group consumed one six-ounce yogurt each day for the duration of the twelve-week intervention. The control group received no yogurt and was instructed to continue their normal dietary patterns, while avoiding a given list of fermented foods, including yogurt. The study took place in four geographical locations: Coeur d'Alene, Idaho; Boise, Idaho; Blackfoot, Idaho; and Manhattan, Kansas. All study procedures were reviewed and approved by the University of Idaho's Institutional Review Board.

### *Laboratory Visits*

Participants attended two testing meetings, one at baseline and one as a follow-up, in a comfortable, private environment at each testing site. Test visit one was scheduled before the intervention began, and test visit two upon completion of the twelve-week intervention. At baseline, participants completed a demographic health questionnaire and a customized cognitive battery using the National Institute of Health (NIH) Toolbox® for Assessment of Neurological and Behavioral Function (Weintraub, et al., 2013). The cognitive test battery was completed again once the intervention was complete. Researchers were trained to administer baseline and post-tests in a consistent manner throughout each geographic location.

### *Tests*

The National Institute of Health Toolbox tests have been developed and validated for use in research (Weintraub et al., 2013). The NIH Toolbox contains assessments for four domains of neurological and behavioral function, including cognition, motor, sensation, and emotion. For the purposes of this study, only the cognition portion will be discussed. The

cognitive portion of the NIH Toolbox was designed to provide consistency and convenience within research that focuses on cognitive performance in epidemiological studies and clinical trials (Weintraub et al., 2013). Within the cognition test, there were five "games" or tests that each participant performed which are measured fluid cognitive abilities. The tests included the Flanker and Inhibitory Control and Attention Test, the Dimensional Change Card Sort Test, the Picture Sequence Memory Test, the List Sorting Working Memory Test, and the Pattern Comparison Test. The tests, overall, measure executive function, attention, episodic memory, processing speed, and working memory.

According to the NIH Toolbox Scoring and Interpretation Guide by Slotkin and Colleagues (2012), the Flanker and Inhibitory Control and Attention Test assesses the participant's attention and inhibitory control. The test requires the subject to concentrate on a given stimulus while inhibiting attention to a distractor. This involves a row of arrows, the middle arrow being the intended center of focus, and a row of arrows beside it as the distractors. The participants choose which direction the middle arrow is pointing, while the arrows "flanking it" could be pointing the same direction as the middle arrow, or a different direction. This requires the participant to focus on relevant stimuli and ignore irrelevant distractors. The test measures executive function, and as previously mentioned, more specifically measures inhibitory control and attention. The test is scored based on the participant's reaction time and accuracy. For this test, the Flanker Computed Score is calculated, which provides a score indicating the amount of improvement or decline in between two tests (the baseline cognition test and the post-test).

The Dimensional Change Card Sort Test provides a measure of executive function, and more specifically, of cognitive flexibility. In this test, two pictures are shown, for example, a yellow ball and a blue truck. The participants are asked to match test pictures by shape (ball or truck) or by color (blue or yellow). Initially, the test will only ask participants to match test picture solely by shape and then solely by color, then the test will combine the two. Similar to the Flanker and Inhibitory Control and Attention Test, a Dimensional Change Card Sort Test Computed Score is provided.

The Picture Sequence Memory Test provides a measurement for episodic memory. This test involves showing the participant a series of pictures, presented and narrated in a specific order. The participants are asked to recall the pictures back into the same sequence as

they were presented. This is demonstrated over two testing trials – the first containing only six pictures, and the second trial containing up to eighteen pictures. Scoring for this test is based on how many pictures the participant correctly places adjacent to each other as initially presented. The Picture Sequence Memory Test Computed Score is provided for this test.

The List Sorting Working Memory Test measures working memory, focusing on information processing and storage. This involves recalling and sequencing various pictures shown – presented orally and visually. For example, different pictures of foods are shown and presented orally. The participant is then asked to remember them and say them back to the researcher in the size order, from smallest to largest. Initially, the pictures are presented in one dimension only, either as food or as animals. Then the pictures are presented in two dimensions, mixing both food and animals. The test is scored by adding the number of items answered correctly. The List Sorting Raw Score is provided to show improvement or decline over time.

The Pattern Comparison Processing Speed Test measures speed of processing by requiring participants to decide whether two pictures that are shown side-by-side are the same or different. The pictures are designed to be very simple – allowing the test to mainly measure processing speed. The pictures are shown one at a time, and the participant answers as many as possible for ninety seconds. The Picture Sequence Memory Test Computed Score is provided for this test, showing an improvement or decline between two tests.

Additionally, a Cognition Fluid Composite Score is provided. This score includes all tests that involve fluid ability measures (all tests previously mentioned).

### *Baseline and Post-Tests*

The cognitive performance test from the NIH Toolbox was administered at week 0 (pre-intervention) and at the end of week 12 (post-intervention). Testing procedures were consistent at baseline tests and post-tests.

### *Dietary Intervention*

During the intervention, participants in the treatment group were given seven 6-oz. containers of yogurt each week, which contained live probiotics. Participants were instructed to consume one 6-oz. container per day. The brand of yogurt that was used for the treatment group was *Nancy's 100% Grass-fed Vanilla Yogurt*, containing probiotic species

*Bifidobacterium lactis, Lactobacillus acidophilus, Lactobacillus rhamnosus and Lactobacillus casei*.

At week's end, the researchers collected all seven of the participant's weekly yogurt containers to confirm that all were consumed during the previous week. The researcher also collected a weekly yogurt log filled out by each participant, indicating the time and day of yogurt consumption along with the other foods that were consumed with the yogurt. Participants received the next seven containers for the following week at this time. This continued for the duration of the 12-week study.

## Participants

Forty-seven healthy adults, both male and female, age ≥ 50, were selected from Boise, Idaho; Coeur d'Alene, Idaho; Blackfoot, Idaho and Manhattan, Kansas. In each location, there was a treatment group and a control group, with a total of n=24 for the treatment group and n=23 for the control group. Written consent was given by participants prior to data collection. All participants were competent to give consent.

Exclusion data included: (1) currently taking probiotic supplements, (2) lactose intolerance (only excluded from treatment group), (3) known dairy allergy (only excluded from treatment group). Exclusion information was screened for upon recruitment and verified at the baseline meeting.

## Recruitment, Retention, and Compliance

To find participants within the target age group (≥ 50 years old), volunteers were recruited from local senior centers, community health sites, and other extension programs recruiting older adults using flyers and through word of mouth. All participants were compensated with a total of $50 for completing the study. Participants were given $10 after the initial tests for their time at the baseline meeting, and a $40 were given to participants for their time in the 12-week intervention and follow-up testing. Compliance within the treatment group was determined through count of empty yogurt containers and consumption logs indicating participants consumed yogurt every day for the 12-week intervention. Subjects were expected to continue eating their normal diet in addition to the daily yogurt supplement provided by the researcher. Compliance within the control group involved the avoidance of fermented foods from the provided "fermented foods to avoid" list for the 12-week period and

was assessed by a log form where participants were asked to record any time they consumed one of the fermented foods on the list.

## Statistical Analyses

Cognition scores were analyzed separately based on scores from the NIH toolbox test, completed at the preliminary and end-of-study testing meetings. For the purpose of the study, computed scores were analyzed for the Flanker Inhibitory Control and Attention Test, Dimensional Change Card Sort Test, Pattern Comparison Processing Speed Test, and the Picture Sequence Memory Test. For the List Sorting Working Memory Test, raw scores were used. For the Fluid Cognition Composite Scores, the uncorrected standard score was used. The SAS software (Statistical Analysis Software, Version 9.4) was used for data analysis. An analysis of variance (ANOVA) procedure was used to compare the change in scores from the baseline and follow-up tests between groups as well as the change from pre to post within groups. An alpha value of 0.05 was set to determine significance.

## Results

### *Participant Characteristics*

Participant characteristics are shown in table 3.1. Forty-seven participants completed this study (n=23 for control, n=24 for treatment). Of the participants that completed the study, 28 were female (59.6%) and 19 were male (40.4%). The control group contained 12 females (52.2%) and 11 males (47.8%). The treatment group contained 16 females (66.7%) and 8 males (33.3%). The participant's age ranged from 50 to 98 years (M=70.1). The majority (97.9%) of participants graduated high school and over half of the participants had attended at least two years of college at a four-year program. Over one-third (36%) of participants had a bachelor's degree or higher. As previously mentioned, the study took place in four geographical locations. Four participants were located in Blackfoot, Idaho; ten participants in Manhattan, Kansas; nineteen in Coeur d'Alene, Idaho; and fourteen in Boise, Idaho.

Participant compliance was tracked using weekly yogurt logs and collection of empty yogurt containers. Average compliance among the treatment group was 87%. Of the 52 participants recruited, five did not complete the study. Four of the participants were recorded as dropouts for reasons being an unrelated illness (two participants), other medical complications (one participant), and time conflicts (one participant). The other participant was

recorded as a loss to follow-up (did not show up for the final assessments and did not return calls or messages).

| **Table 3.1: Participant Characteristics** | | | |
|---|---|---|---|
| | **Yogurt** | **Control** | **Total** |
| **Sample size** | 24 | 23 | 47 |
| **Age (years) (M)** | 70.4 | 69.8 | 70.1 |
| **Gender (%)** | | | |
| *Male* | 33 | 48 | 40 |
| *Female* | 67 | 52 | 60 |
| **Education (%)** | | | |
| *High school graduate* | 13 | 13 | 13 |
| *Some college* | 46 | 57 | 51 |
| *Bachelor's degree* | 13 | 13 | 13 |
| *Master's degree or higher* | 29 | 17 | 23 |
| **Race (%)** | | | |
| *American Indian or Alaska Native* | 0 | 4 | 2 |
| *Asian* | 0 | 17 | 9 |
| *White* | 86 | 48 | 67 |
| *Hispanic or Latino* | 0 | 9 | 4 |
| *Non-Hispanic or Non-Latino* | 9 | 22 | 16 |
| *Other* | 5 | 0 | 2 |

*Cognitive Test Outcomes*

Table 3.2 represents the results for the analysis of variance of effect of group on change in cognition. In order to determine if there was a difference between baseline test scores and post-test scores in the treatment group compared to the control group, a one-way analysis of variance was done and indicated a significant difference between groups for the Dimensional Change Card Sort Test ($F=4.54$, $p=0.04$). This difference remained significant when adjusting for age ($F=4.43$, $p=0.04$), but was attenuated when adjusting for education or sex. There was no clear evidence of a difference between groups for the Fluid Cognition Composite Scores, Flanker Inhibitory Control and Attention Test, List Sorting Working Memory Test, Pattern Comparison Processing Speed Test, and the Picture Sequence Memory test in any of the four models (univariate, age-adjusted, education adjusted, or sex adjusted).

Table 3.3 represents the results for the analysis of variance of change in cognition within groups. In order to determine the difference between baseline test scores and post-test scores within the treatment group and the control group, a one-way analysis of variance was done and indicated no clear evidence of a difference within groups for the Fluid Cognition Composite Scores, Flanker Inhibitory Control and Attention Test, List Sorting Working

Memory Test, Dimensional Change Card Sort Test, Pattern Comparison Processing Speed Test, and the Picture Sequence Memory test. Results remained non-significant for all cognitive outcomes when adjusting for age, education, or sex.

| Table 3.2: Analysis of Variance of Effect of Group on Change in Cognition | | |
|---|---|---|
| **Cognitive Assessment** | **F-Value** | **p-value** |
| **Cognition Fluid Composite** | | |
| Univariate | 0.04 | 0.85 |
| Age-Adjusted | 0.02 | 0.89 |
| Education-Adjusted | 0.92 | 0.34 |
| Sex-Adjusted | 0.80 | 0.38 |
| **Flanker Inhibitory Control and Attention** | | |
| Univariate | 0.53 | 0.47 |
| Age-Adjusted | 0.50 | 0.48 |
| Education-Adjusted | 0.62 | 0.44 |
| Sex-Adjusted | 0.37 | 0.55 |
| **List Sorting Working Memory** | | |
| Univariate | 0.47 | 0.49 |
| Age-Adjusted | 0.43 | 0.51 |
| Education-Adjusted | 0.85 | 0.36 |
| Sex-Adjusted | 0.41 | 0.52 |
| **Dimensional Change Card Sort** | | |
| Univariate | 4.54 | 0.04 |
| Age-Adjusted | 4.43 | 0.04 |
| Education-Adjusted | 1.14 | 0.29 |
| Sex-Adjusted | 3.70 | 0.06 |
| **Pattern Comparison Processing Speed** | | |
| Univariate | 0.97 | 0.33 |
| Age-Adjusted | 1.58 | 0.22 |
| Education-Adjusted | 0.66 | 0.42 |
| Sex-Adjusted | 0.80 | 0.38 |
| **Picture Sequence Memory** | | |
| Univariate | 0.09 | 0.77 |
| Age-Adjusted | 0.12 | 0.74 |
| Education-Adjusted | 0.41 | 0.52 |
| Sex-Adjusted | 0.02 | 0.89 |

| Table 3.3: Analysis of Variance of Change in Cognition within Group | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Yogurt Group | | | | Control Group | | | |
| **Cognitive Assessment** | **Estimate** | **StdErr** | **t-value** | **p-value** | **Estimate** | **StdErr** | **t-value** | **p-value** |
| **Cognition Fluid Composite** | | | | | | | | |
| Univariate | 2.08 | 1.43 | 1.46 | 0.15 | 2.48 | 1.46 | 1.70 | 0.10 |
| Age-Adjusted | 2.15 | 1.34 | 1.60 | 0.12 | 2.41 | 1.37 | 1.76 | 0.09 |
| Education-Adjusted | 2.08 | 1.43 | 1.45 | 0.15 | 2.46 | 1.46 | 1.68 | .10 |
| Sex-Adjusted | 3.44 | 1.94 | 1.78 | 0.08 | 1.01 | 1.92 | 0.53 | 0.60 |
| **Flanker Inhibitory Control and Attention** | | | | | | | | |
| Univariate | -0.01 | 0.14 | -0.05 | 0.96 | 0.14 | 0.15 | 0.98 | 0.33 |
| Age-Adjusted | -0.00 | 0.14 | -0.00 | 0.99 | 0.14 | 0.14 | 0.99 | 0.33 |
| Education-Adjusted | -0.01 | 0.14 | -0.04 | 0.97 | 0.14 | 0.14 | 0.96 | 0.34 |
| Sex-Adjusted | 0.02 | 0.15 | 0.14 | 0.89 | 0.15 | 0.15 | 1.00 | 0.33 |
| **List Sorting Working Memory** | | | | | | | | |
| Univariate | 0.21 | 0.63 | 0.33 | 0.74 | 0.83 | 0.64 | 1.29 | 0.20 |
| Age-Adjusted | 0.23 | 0.62 | 0.37 | 0.71 | 0.81 | 0.63 | 1.28 | 0.21 |
| Education-Adjusted | 0.21 | 0.64 | 0.33 | 0.75 | 0.82 | 0.65 | 1.26 | 0.21 |
| Sex-Adjusted | 0.24 | 0.65 | 0.37 | 0.71 | 0.83 | 0.65 | 1.28 | 0.21 |
| **Dimensional Change Card Sort** | | | | | | | | |
| Univariate | -0.14 | 0.13 | -1.15 | 0.26 | 0.24 | 0.13 | 1.86 | 0.07 |
| Age-Adjusted | -0.14 | 0.13 | -1.14 | 0.26 | 0.24 | 0.13 | 1.83 | 0.07 |
| Education-Adjusted | -0.14 | 0.13 | -1.13 | 0.27 | 0.24 | 0.13 | 1.81 | 0.08 |
| Sex-Adjusted | -.10 | 0.13 | -0.80 | 0.43 | 0.24 | 0.13 | 1.92 | 0.06 |
| **Pattern Comparison Processing Speed** | | | | | | | | |
| Univariate | 3.67 | 1.86 | 1.97 | 0.06 | 1.04 | 1.90 | 0.55 | 0.59 |
| Age-Adjusted | 4.00 | 2.20 | 1.82 | 0.08 | -0.58 | 2.51 | -0.23 | 0.82 |
| Education-Adjusted | 3.66 | 1.86 | 1.97 | 0.06 | 1.01 | 1.90 | 0.53 | 0.60 |
| Sex-Adjusted | 3.44 | 1.94 | 1.78 | 0.08 | 1.01 | 1.92 | 0.53 | 0.60 |
| **Picture Sequence Memory** | | | | | | | | |
| Univariate | 16.41 | 13.08 | 1.25 | 0.22 | 10.96 | 13.37 | 0.82 | 0.42 |
| Age-Adjusted | 23.21 | 17.11 | 1.36 | 0.19 | 13.55 | 19.55 | 0.69 | 0.50 |
| Education-Adjusted | 16.48 | 13.33 | 1.24 | 0.22 | 11.00 | 13.61 | 0.81 | 0.42 |
| Sex-Adjusted | 13.02 | 13.45 | 0.97 | 0.34 | 10.51 | 13.35 | 0.79 | 0.44 |

## Discussion

This pilot study found that daily consumption of dairy-based probiotics for 12-weeks had no clear evidence of a change in cognitive test outcomes in older adults when compared to a control group. While a difference between groups was found in the Dimensional Change Card Sort test (measuring cognitive flexibility) for the univariate and age-adjusted model, the control group unexpectedly had a more substantial change in test scores from baseline to post-test compared to the control group. This result may be due to chance, or potentially due to the practice effect, as shown in a study by Basso (2001), in which participants were administered a card-sorting test two times, and scored higher in the second card-sorting test due to having practiced the test once before. More research is needed to address this unforeseen outcome.

While other studies looking at the effects of probiotic supplementation and cognitive function exist (Okon-Singer, et al., 2015; Akbari, et al., 2016; Chung, et al., 2014; Hogervorst, et al., 2011), this is the first study to our knowledge to look at the effects of probiotic consumption in the form of yogurt on cognitive outcomes in older adults. Research studies looking directly at the effects of probiotics on brain function is limited, however, it is thought that diet may have a direct impact on the gut microbiome (Abraham, et al., 2016; Beilharz et al., 2017). Increasing amounts of research indicate that the gut microbiome, and hence, probiotic consumption, may impact cognitive function (Okon-Singer, et al., 2015; Akbari, et al., 2016; Chung, et al., 2014; Hogervorst, et al., 2011). While results are mixed, studies have found promising results that warrant further research.

Akbari and colleagues found that cognitive function was improved in thirty older adults with AD (ages 60-90 years old) by supplementing probiotic milk. While the population age group is similar to the current study, neurodegenerative diseases were not screened for in the current study, but the participant sample was not intended to target those with AD. Similar to the current study, this included a 12-week intervention. The probiotic milk contained the species *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Lactobacillus fermentum*, which includes the use of one consistent species of probiotics with the current study (*Lactobacillus acidophilus*). The probiotics were administered in the amount of $2 \times 10^9$ CFU/g for each. The concentration of the probiotics used in the yogurt in the current study does not specify CFU/g for each strain, making it difficult to compare concentrations. However, the yogurt product states that it contains 41 billion live probiotics

per serving. This specific probiotic intervention included the daily consumption of 200 milliliters of probiotic milk for a 12-week period and the results showed an improvement in cognitive test scores in the treatment group compared to a control group (Akbari, et al., 2016). Sample sizes of the current study and the above-mentioned study are similar, with n=24 for the treatment group of the current study and n=30 for Akbari and colleague's study. Another differentiating factor is the type of tests used to assess cognitive function. The mini-mental state examination was used in the study assessing AD patients, which is a shorter assessment originally developed for evaluation of cognitive impairment and global cognition (Monroe & Carter, 2012). As mentioned previously, the NIH Toolbox was used in the current study to assess areas of cognition that may be impacted by diet (fluid cognition) and to get a more comprehensive and specific measure of cognition. Differences in cognitive function tests used, concentration and species of probiotics administered, and participant population may be factors explaining why the current study saw minimal results, while the above-mentioned study saw significant improvements in cognitive function tests.

Another study by Chung, et al., (2014) looked at the effects of a 12-week fermented milk intervention on cognitive function in older adults (ages 60-75 years old) and found an improvement in neuropsychology test scores in the treatment group compared to the control group. The neuropsychology tests administered included the digital-span test, story recall test, and verbal-learning test. Overall, these tests measured attention, working memory, short-term memory, and long-term memory. Tests such as the rapid-visual information-processing task, Stroop color-word test, and serial 3s and 7s test were grouped together and administered to measure cognitive fatigue. Although different cognitive tests were used, the use of a battery of cognitive tests to assess a variety of similar cognitive domains is consistent with the current study. Like the current study, Chung and colleague's study included a 12-week long intervention. However, the probiotic intervention differed, as it included oral administration of the species *Lactobacillus helveticus* only, with daily doses of 125, 250, and 500 milligrams. Dosages are difficult to compare, as the study does not specify the colony-forming units in the tablets administered, only the mass in milligrams of the tablet administered. Unlike the current study, probiotics were administered in the form of a supplement (tablets) rather than in food containing probiotics. Participants in each of the three groups took a tablet containing the previously mentioned amounts four times daily. The placebo group was instructed to do the

same, taking tablets that were identical in color, shape, and size, but contained no probiotics. A total of 36 participants completed the study with n=10 for the placebo group, n=10 for the 500-milligram group, n=7 for the 1000-milligram group, then n=9 for the 2000-milligram group. The results of the study showed an improvement in the treatment groups for story recall, verbal-learning test scores, some items in the Stroop color-word test and serial 3s and 7s test, indicating that the intervention improved mainly attention and working memory in participants (Chung, et al., 2014). This warrants further research as the current study found no effect on tests measuring working memory and attention after the 12-week probiotic intervention. It is possible, however, that the lack of results seen in the current study are due to differences mentioned above, such as the species of probiotics and dosages administered in the intervention. The use of the isolated species of *Lactobacillus helveticus* in this study shows promising results that this specific probiotic may have positive impacts on cognitive function. In a future study, the administration of the species *Lactobacillus helveticus* would be important to explore further.

A positive association was found between fermented food consumption (tofu and tempeh) and cognitive function in older adults in a cross-sectional study done by Hogervorst and colleagues (2011). Cognitive data for 142 participants (ages 56-97 years old) were used from a previous study (Hogervorst, et al., 2008) to analyze the relationship between tofu and tempeh consumption (using a Food Frequency Questionnaire) with cognitive function scores (using a word learning test sensitive to dementia). Consumption of food containing probiotics was evaluated rather than probiotics administered in tablet or supplement form, comparable to the current study. Since fermented foods like tempeh may contain a variety of probiotic species, it is not entirely known which species or dosages were consumed by participants. However, it is believed that probiotics from the genera *Lactobacillus, Streptococcus*, and *Leuconostoc* are predominant in fermented foods such as tempeh, so it is possible that participants consumed similar species of *Lactobacillus* as the current study. (Rezac, Kok, Heermann, & Hutkins, 2018). The results of the study showed a positive linear association indicating that the participants who had a diet that consisted of a higher tofu and tempeh consumption scored higher in immediate recall tests than those who consumed less tofu or tempeh. The positive association in cognition was only found in a group of participants with an average age of 67 (less than 73 years of age). There was no positive association between

tofu or tempeh consumption in the group with an average age of 80 years old (Hogervorst, et al., 2011). This indicates a need for future research targeting more specific age groups to indicate the effect of diet on cognitive function. It should also be noted that this study was intended to determine if tofu and tempeh have an effect on cognitive function, specifically looking at the plant estrogens and isoflavones in soy rather than probiotic content. However, fermented foods such as tempeh and tofu contain probiotic species and the results of the study support the theory that cognition can be impacted by diet. Unlike the current study, this is a cross-sectional study, indicating that the amount of time the participants have been consuming this type of diet is unknown and warrants future studies with longer interventions.

The current study indicated that daily dairy-based probiotic consumption over 12-weeks had negligible effects on cognitive outcomes in older adults (ages 60-98 years old). Considering the previously mentioned studies involving probiotic consumption and cognitive function, the results are mixed, indicating a need for future research. Direction for future studies should target specific age groups such as middle-aged and young-old adults, include larger sample sizes, and include fecal samples to confirm gut microbiome changes. Administration of different probiotic species, strains, and concentrations should be explored. The use of the NIH Toolbox is also encouraged, as this tool was developed to create consistency among research studies assessing cognitive function and encompasses a wide variety of cognitive abilities.

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