A META-ANALYSIS ON THE CORRELATION BETWEEN DYSBIOSIS AND MENTAL HEALTH: THE EFFECTS OF PROBIOTICS/PREBIOTICS/SYNBIOTICS ON MEMORY, WITH A SPECIFIC FOCUS ON ALZHEIMER'S DISEASE

by

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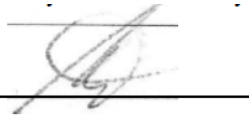
DECLARATION

I, Neeske Nel, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of MSc (Med) Pharmacotherapy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of candidate)

16 day of March 2021 in Bethlehem

Do not go gentle into that good night,

Old age should burn and rave at close of day;

Rage, rage against the dying of the light.

–Dylan Thomas

ABSTRACT/SUMMARY

Alzheimer's disease (AD) influences the elderly in all societies of the world, both due to its prevalence and influence on quality of life. Considering that no cure is currently available and several theories regarding the pathogenesis of Alzheimer's disease exist, it is worth considering the use of supplementary probiotics/ prebiotics/ synbiotics due to the possibility of a gut-brain link. This research attempted to find more certainty about the use of probiotics/ prebiotics/ synbiotics as a supplementary treatment for memory impairment – specifically in AD/dementia – from already existing studies. Data was pooled using a random effects meta-analysis to find the overall outcomes from included studies for total cognition as well as subsets of neurocognitive functions. The overall results for short term and delayed memory proved a small but significantly improved standardized mean difference of experimental outcomes compared to control outcomes, whereas the total cognition scores were significant, but had too much heterogeneity among the included study results, indicating inconsistency of intervention effects across studies. It was concluded that ingestion of microbial supplements may be beneficial as supplementary treatment for AD patients, but larger scale studies are needed.

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Nomenclature/List of abbreviations

8-OHdG – 8-hydroxy-2'-deoxyguanosine
Aβ – amyloid-β
AD – Alzheimer's disease
ATP – adenosine triphosphate
BDNF – brain-derived neurotrophic factor
CANTAB – Cambridge Neuropsychological Test Automated Battery
CBB – CogState brief battery
CERAD – Consortium to Establish a Registry for Alzheimer's Disease
CSF – cerebrospinal fluid
CVLT – California Verbal Learning Test
FDG – fluorodeoxyglucose
FOS - fructooligosaccharide
GABA – gamma aminobutyric acid
GIT – gastrointestinal tract
GOS – galactooligosaccharide
HD – Huntington's disease
IBD – inflammatory bowel disease
LPS – lipopolysaccharides
MCI – Mild Cognitive Impairment
MD – mean difference
MetS – metabolic syndrome
MMSE - Mini-Mental State Exam
MoCA – Montreal Cognitive Assessment
MRI – magnetic resonance imaging
NSAIDs – non-steroidal anti-inflammatory drugs
PAL – paired associate learning test
PET – positron emission tomography
p-Tau – phosphorylated tau proteins
RAVLT – Rey Auditory-Verbal Learning Test
RBANS – Repeatable Battery for the Assessment of Neuropsychological Status
RevMan – Review Manager 5.4 program
ROS – reactive oxygen species
SCFAs – short-chain fatty acids
SD – standard deviation
SEM – standard error of mean
SMD – standardized mean difference
TNF-α – tumor necrosis factor
TYM – Test Your Memory
VLT – Verbal Learning Test
WAIS – Wechsler's Adult Intelligence Scale

1. BACKGROUND

1.1. The link between Alzheimer's and the gut

1.1.1 Alzheimer's disease (AD)

Modern medicine has prolonged life expectancy and with it, increased frequency of geriatric diseases. Diseases associated with old age create challenges such as having to reduce symptoms of illness, decrease disease complications and minimalize risk factors. One of these geriatric diseases is dementia, growing exponentially in prevalence with age, which greatly burdens families and society (Lin *et al*., 2019). An estimated 50 million individual lives are affected by dementia globally (Luca *et al*., 2019), with a prevalence around 0.7-1.8% in the populace aged 60 to 64 years, and 28.7-63.9% in people aged over 90 years (Prince *et al*., 2013). The most frequent form of dementia is Alzheimer's disease (AD), especially in the elderly, accounting for 50-60% of all cases (Luca *et al*., 2019).

AD is a multifactorial neurodegenerative condition that has an effect on the central and peripheral nervous system (Lin *et al*., 2019). Biomarker research has allowed for AD to be diagnosed as a gradually progressive chronic brain illness that can be detected years before any clinical symptoms appear. Years (even decades) before the manifestation of behavioral changes and cognitive decline, the pathological characteristics of AD (including extracellular amyloid-*β* (Aβ) plaques, hippocampus atrophy and intracellular neurofibrillary tangles of hyper phosphorylated tau protein) can be recognized (Luca *et al.,* 2019). For clinical diagnosis, the biomarker framework has been set, including cerebrospinal fluid (CSF) levels of total tau and phosphorylated tau (p-Tau), amyloid b-42 (Ab42) level, Fluorodeoxyglucose (FDG) uptake on positron emission tomography (PET), amyloid imaging with PET, and structural magnetic resonance imaging (MRI) (Vemuri & Jack, 2010). One of the characteristics of AD is reduced levels of insulin activity in the brain, defining AD as a brain-specific diabetes. The reduction of functionally accessible neuronal insulin receptors due to Aβ and tau accumulation may be one of the underlying mechanisms of central insulin resistance (Pistollato *et al*., 2018).

The risk for developing AD and other neurocognitive impairments is primarily linked to ageing. Metabolic syndrome (MetS), obesity, insulin resistance, type 2 diabetes

and cardiac risk factors are secondary factors in the etiopathogenesis and establishment of AD. These disorders all have highly comparable molecular and metabolic features. Patients with AD often present with dyslipidaemia, impaired glycaemic control, deregulated glucocorticoid levels, oxidative stress, chronic inflammation and mitochondrial metabolism impairments (Pistollato *et al*., 2018). Typically, early symptoms of AD include intermittent memory impairment followed by other deficits in cognitive functions (e.g. attention, visuospatial, language, and decision-making functions), eventually leading to a loss of facilities needed for day-to-day living and dementia (Luca *et al*., 2019).

Although determinations to target the pathological hallmarks of AD have been attempted, a cure remains elusive. The disproportionately high Aβ production relative to clearance causes accumulation, which causes neuronal dysfunction. Neuro-inflammation may hinder Aβ clearance and further the pathology of AD (Lin *et al*., 2019). The accumulation of Aβ and p-Tau proteins interferes with brain function. Excessive Aβ production causes mitochondrial damage, depletion of adenosine triphosphate (ATP) and an increase of reactive oxygen species (ROS), which may lead to axonal transport and neurotransmission impairments (Pistollato *et al.,* 2018). It also triggers the inflammatory activity of microglia, leading to further Aβ accumulation, neuronal debris collection and the continuous manufacturing of pro-inflammatory cytokines and ROS, causing chronic inflammation. The presence of microglia cells and increased pro-inflammatory cytokine levels (TNF-α and/or interleukin-6) in the brain tissue and serum of AD patients substantiates that neuro-inflammation has as much of a causal role in AD pathogenesis as the plaques and tangles (Wong *et al*., 2018). Also, systemic inflammation may speed up the pathology of AD (Krstic *et al*., 2012), which is substantiated by epidemiological studies that show a lower risk for developing dementia in users of non-steroidal anti-inflammatory drugs (NSAIDs) (Vlad *et al.,* 2008). Inflammation may, in fact, play a vital part in numerous chronic disorders, like type 2 diabetes, depression and AD (Luca *et al*., 2019).

Another consideration in AD pathology is oxidative stress (an imbalance of ROS production and the antioxidant defence system, causing subsequent oxidative

damage) (Wong *et al*., 2018). Dyslipidaemia and impaired glycaemia can increase the lipid peroxidation levels, progressively causing impaired antioxidant reactions and resulting in oxidative metabolism and eventual neuronal damage (Pistollato *et al*., 2018). Oxidative stress signifies the method employed by Aβ and p-Tau proteins to facilitate the AD pathology and cause synaptic impairment, neuro-inflammation, neuronal apoptosis and neurotransmitter imbalance. Oxidative stress may enhance tau hyperphosphorylation and the production and deposition of Aβ, creating a continuous loop that supports the inception and progression of AD (Wong *et al*., 2018).

1.1.2 Gut-brain axis

The human gastrointestinal tract (GIT) is the biggest reservoir of microbial genomes with the microbiota consisting of over 100 trillion microorganisms with at least 1000 different bacterial species, bacteriophage particles, viruses, fungi, and archaea (Wong *et al*., 2018). This microbiota plays an elaborate part in its host's physiological and pathophysiological functions and, in cases of healthy individuals, constitutes an ecological community which benefits the host (Luca *et al*., 2019). In healthy individuals the GIT microbiota lives in eubiosis, creating a helpful co-existence with the host by contributing to regulating the barrier effect, metabolism (including drug metabolism), immune competency and tolerance and affecting the synthesis of various substances, e.g. neurotransmitters (Iebba *et al*., 2016).

The gut-brain axis network consists of the GIT, CNS, enteric nervous system, autonomic nervous system, neuroendocrine system and immune system (Wong *et al*., 2018). The microbiota-gut-brain axis is the bidirectional communication between the GIT microbiota and the CNS through multiple intersecting pathways, including neural, immune, endocrine, and metabolic signals (Lin *et al*., 2019), as well as neuromodulatory molecules and bacterial metabolites (Luca *et al*., 2019). The brain converses with the GIT by moderating permeability, secretion, motility and immunity. Simultaneously the GIT can influence brain function and behaviour (Wong *et al*., 2018). Numerous bacteria produce neurotransmitters, including serotonin and gamma-aminobutyric acid (GABA), directly. *Lactobacillus rhamnosus* may benefit

the treatment of mood disorders by regulating the neural GABA receptor expression (Luca *et al.*, 2019). Modifications in the GIT microbiota configuration may lead to imbalanced gut homeostasis and have harmful effects on the CNS (Wong *et al*., 2018), as the GIT microbiota is firmly intertwined with the gut and the brain (Lin *et al.*, 2019). The GIT microbiota could play an essential part in the pathogenesis of chronic neurodegenerative diseases such as diabetes, depression and AD (Luca *et al*., 2019).

1.1.3. The normal, aging and AD human GIT

1.1.3.1 Normal human GIT

The migration of microbes to the GIT occurs during birth. The formation of the gut biome is highly dynamic during infancy and resembles adult organization around three years of age (Vogt *et al.*, 2017). Thereafter, the bacterial composition tends to stay stable in any given individual, assuming an absence of pathologic states (Boyle *et al*., 2006), although with significant interpersonal variation, especially in elderly individuals (Vogt *et al.*, 2017). The GIT microbiota composition may differ between healthy and unhealthy individuals (Lopetuso *et al*., 2018) and, in spite of the difficulty in defining a healthy microbiota, it is suggested that a healthy adult microbiota sustains community stability and a diversity of species (Luca *et al*., 2019).

The normal human microbiome consists of some eukaryotic fungi, archaea, protists and bacteria. Bacteria make up the majority of the microbiome and are the most noticeable components. A normal human adult harbors around 10^{12} bacteria on the skin, 10^{10} in the mouth, and 10^{14} in the GIT. The human GIT houses hundreds of diverse bacterial species – far more than the eukaryotic cells that make up a human (Kenneth, 2012). The Firmicutes (e.g. Lactobacillus) and the Bacteroides characterize the core bacterial phyla of the gut, succeeded by Actinobacteria (e.g. Bifidobacterium), Proteobacteria and Cyanobacteria (Luca *et al*., 2019). The human esophagus contains only the bacteria swallowed with food and saliva. Normally few bacteria, primarily acid-tolerant Lactobacilli (and frequently the pathogenic *Helicobacter pylori*), are cultured from the stomach, due to the high acidity. The proximal small intestine has a comparatively scant Gram-positive flora, about 10^{5} -

10^7 bacteria per ml of fluid, mostly containing Lactobacilli and *Enterococcus faecalis*. The distal small intestine houses more bacteria (10^8/ml) and a greater variety of species, including coliforms (such as *E. coli*), Bacteroides, Lactobacilli and Enterococci (Kenneth, 2012). The highest bacterial concentration is in the colon (up to 10^{11} CFU/g) (Boyle *et al*., 2006), with by far the most of this microbial populace located in the distal gut (Vogt *et al.*, 2017). The colon's microbiome composition is qualitatively alike to that found in feces: coliforms and the anaerobic species Bacteroides and Bifidobacteria (*Bifidobacterium bifidum*) are prominent. The colon also regularly contains Enterococci, Clostridia, Lactobacilli and, sometimes, significant numbers of anaerobic methanogens (up to 10^{10}/gm). The greatest influence of the bacterial microbiota on its host is observed in the intestinal tract (Kenneth, 2012). The GIT microbes complete crucial functions for human health such as energy extraction, vitamin biosynthesis, shielding against pathogen establishment and infection, and development and the activity of the immune system (Vogt *et al.*, 2017).

Differences in the human gut microbiota composition are influenced by genetics, gender, age, stress, diet, nutrition, cultural circumstances and antibiotics usage – which greatly disturb the microbiota composition (Kenneth, 2012). A variety of GIT and metabolic diseases are linked to changes in the balance of this multifaceted ecosystem, including obesity, inflammatory bowel disease and insulin resistance. Modifications in the GIT microbiome have also been linked to neurological conditions, including multiple sclerosis, autism spectrum disorder, AD and Parkinson's disease (Vogt *et al.*, 2017). There are, however, indications that probiotic bacteria can significantly affect a healthy GIT microbiota composition (Boyle *et al.,* 2006).

1.1.3.2 Aging human GIT

The aging process affects the GIT microbiota with regard to composition and functionality. In a type of self-sustaining loop, the aged-type microbiota contributes to numerous age related phenomena like immunosenescence (the steady deterioration of the immune system owed to natural aging) and inflammaging (chronic low-grade inflammation that progresses with advanced age) (Biagi *et al*., 2011). Ageing may also influence microbial diversity. Biological age (not

chronological age) correlates with decreased microbial diversity in stool samples. Pathological ageing is linked to a decrease in microbial diversity, in comparison to a more diverse microbiota in healthy ageing (Toribio-Mateas, 2018). Ageing prompts numerous modifications in the human colonic microbiota, most significantly a decrease in Bifidobacteria, which is a genus that promotes health (Vulevic *et al.*, 2015). The increased proportion of opportunistic bacteria in an aged-type GIT microbiota is one of its most prominent and common characteristics. Therefore, people who live together could influence each other's GIT microbiota composition – especially aging people, who have less resilient microbiota – leading to the results obtained by studies investigating the GIT microbiota of elderly people that are living or hospitalized together possibly being biased due to the shared living environment. The ability of an aging human to form a symbiotic relationship with a gut microbiota altered by age may be among the features that contribute to the host's tendency to reach longevity (Biagi *et al*., 2011).

1.1.3.3 GIT dysbiosis and AD

Gastrointestinal microbiota changes may influence the development and progression of neurological disease, like AD, although what exactly causes dysbiosis and its influence on AD remains unknown (Wong *et al*., 2018). There is a strong relationship between factors like diet and lifestyle and the inception and consolidation of AD (Pistollato *et al.*, 2018). Factors like the abuse of antibiotics or anti-acids, impairment of the immune system or adjustment of the GIT mucosal barrier integrity can modify the balance of the GIT biome and produce a pathological GIT ecosystem, i.e. dysbiosis (Franceschi *et al*., 2019), referring to the imbalance of microbes on or inside the body (Lin *et al.*, 2019). Whereas an eubiotic GIT microbiota plays a positive part in the reduction of ROS, dysbiosis can cause systemic inflammation that can ultimately lead to intestinal infection, IBD (McIlroy *et al*., 2018), microglia activation, damage to the blood-brain-barrier and the consequent crossing of pathogens and immune cells (Luca *et al*., 2019). The effects of dysbiosis are strongly connected to the incidence of both GI and extra-GI diseases (Franceschi *et al*., 2019), including disorders such as obesity, asthma and Parkinson's disease (Pindjakova *et al.*, 2017). Dysbiosis also contributes to the

development and maintenance of AD, depression and type 2 diabetes mellitus – diseases that are frequently linked to high comorbidity rates (Luca *et al*., 2019). These diseases are deeply interconnected and (particularly MetS-related risk factors) have a considerable effect on cerebral morphology and physiology, Aβ deposition and plaque build-up. Healthy dietary habits (high ingestion of plant-based foods, antioxidants, probiotics, nuts, and omega-3 polyunsaturated fatty acids, and decreased ingestion of refined sugars, saturated fats, and animal-derived proteins) improve GIT dysbiosis, improve inflammatory and immune responses, reduce insulin resistance and reduce the risk of neurocognitive impairments (Pistollato *et al.,* 2018).

In AD patients the GIT microbiome has a diminished microbial diversity and distinct deviations in bacterial abundance. Phylum- through genus-wide flora differences include decreased Firmicutes, decreased Bifidobacteria and increased levels of Bacteroidetes (Wong *et al*., 2018). The levels of differentially abundant genera also show a correlation with CSF biomarkers of AD. A connection between increased bacterial abundance (for genera that are more plentiful in AD – especially Bacteroides and Blautia) and greater AD pathology exists, with mainly positive relationships between CSF levels of p-tau and p-tau/Aβ42 and bacterial abundance. Correspondingly, for the genera that are lessened in AD, there is a correlation between reduced bacterial abundance and greater AD pathology. In both the above observations, the strongest correlations between CSF AD biomarkers and bacterial abundance were in the same direction regardless of AD diagnosis. Additionally, AD patients may exhibit comparatively decreased Actinobacteria, mostly due to alterations in Bifidobacterium. Decreased Bifidobacteria and increased Bacteroides in AD patients could signify a gut microbial phenotype with a specific tendency for pro-inflammatory bacterial component translocation (Vogt *et al.*, 2017). The reduced microbiota biodiversity, with a relatively abundant Proteobacteria and a decrease of Bifidobacteria, contributes to the incidence of dementia by significantly reducing beneficial short-chain fatty acids (SCFAs) and also by interfering with lipid metabolism. Especially Bifidobacteria might influence cholesterol by reducing its absorption, production and by aiding its faecal elimination (Luca *et al*., 2019), and indirectly, by increasing the serum leptin levels, a hormone linked to long-term potentiation of the hippocampus and prevention of memory impairments (Vogt *et al.*, 2017). Considering that lipids influence the Aβ oligomer production, the preservation

of microbial biodiversity is essential. Probiotic supplements may normalize serum triglyceride levels and improve cognitive performances, thus augmenting the part of the microbiota that works to maintain a balanced lipid metabolism. Another method of protection against cognitive impairment that the microbiota employs is the capacity of Lactobacilli to decrease ammonia concentrations (Luca *et al*., 2019).

Localized intestinal inflammation or dysbiosis can cause colonization of intrinsic pathogens, leading to increased intestinal permeability and systemic inflammation, which can trigger systemic immune reactions that result in development of AD pathologies and cognitive impairment through the neural, metabolic, endocrine and immune pathways (Montacute *et al*., 2017). Opportunistic GIT bacteria can also excrete immunogenic complexes of amyloids, Lipopolysaccharides (LPS), and other microbial exudates (Wong *et al*., 2018). Considering that elderly individuals demonstrate a relationship between inflammation and increased LPS-binding protein (a microbial translocation marker) and the fact that LPS (a significant part of the outer membrane of Gram-negative bacteria) (Vogt *et al.,* 2017) co-localizes with Aβ in amyloid plaques, there is likely translocation of bacteria from the GIT (Wong *et al*., 2018). Lipopolysaccharides can activate the release of pro-inflammatory cytokines and cause systemic inflammation after translocation from the GIT into the systemic circulation. Therefore, a greater abundance of Gram-negative intestinal bacteria (such as Bacteroides) may cause amplified translocation of LPS from the GIT into the systemic circulation, consequently resulting in inflammation or other mechanisms of exacerbation of AD pathology (Vogt *et al.*, 2017). Lipopolysaccharides and the *Escherichia coli* protein were also identified in the blood vessels and brain parenchyma of AD patients. Bacterial derived amyloids that accumulate in the brain may trigger a sequence of downstream events, leading to phagocyte impairment and increased Aβ deposition, causing region specific brain dysfunction (e.g. the hippocampus and the cerebellum) and contributing to the pathogenesis of cognitive damage (Wong *et al*., 2018).

Bacteria have been shown to influence organs, regardless of their distance from the primary site of infection, and cause neurodegeneration via translocation and molecular mimicry mechanisms, promoting inflammation and the accumulation of Aβ in the brain (Levy *et al.*, 2017). Faecal calprotectin (a heterodimer that is made by

pro-inflammatory proteins S100A8 and S100A9 and used as an inflammatory biomarker to gage the incidence and severity of inflammatory bowel diseases) could be valuable in the evaluation of cognitive decline. The S100A9 protein is a recognized biomarker for diagnosing and determining the progression of dementia and AD. Alzheimer's disease patients tend to present with abnormally high (>50 mg/kg) faecal calprotectin concentrations, indicating an increased intestinal permeability (leaky gut) – where a disturbed intestinal barrier function has resulted in faecal calprotectin translocation into the systemic circulation (Toribio-Mateas, 2018).

1.2. The GIT and microbial supplements

1.2.1. Probiotics

Humans have ingested probiotics for centuries in the conviction that they provide health benefits. Persian custom states that Abraham owed his fertility and long life to frequent yogurt ingestion (Boyle *et al*., 2006). There is a very delicate balance of naturally occurring microorganisms in the immune system and the GIT of the human body. Probiotics are beneficial, living microbes alike to the natural microbes in the human GIT. They can amend the microbial ecosystem of the intestine by prompting antimicrobial metabolites, thus enhancing the body's immune response (Swapna *et al.*, 2017). Probiotics can be used in the management of numerous conditions, including lactose intolerance, acute gastroenteritis, obesity, food allergy, Crohn's disease, atopic dermatitis, rheumatoid arthritis, and colon cancer (Chung *et al*., 2014). Probiotics are consumed both to provide a basic nutritional component and for sustaining a healthy balance in the intestinal tract and immune system. These bacteria are typically selected for their potential health benefits and lack of negative side-effects. Probiotic products are commonly available as dietary supplements and incorporated into yogurts, tablets, powders and creams. Dairy products are an effective vehicle for the delivery of probiotics, but the probiotics must also endure and resist enzymes in the GIT (Swapna *et al.*, 2017).

The probiotics' mechanism of action could be a result of their stimulation of phagocytosis, resistance to colonization, production of antimicrobial compounds, anti-mutagenic effects, cytokine production, and effects on enzyme action and

delivery (Swapna *et al.*, 2017). According to Wong *et al*. (2018) probiotic modulation of AD may be attributed to:

- Immune reaction changes – Probiotics can modulate cytokine production, improve circulation, improve natural killer cell, macrophage, T cell and granulocyte function, and improve antibody responses. Probiotics may ameliorate AD symptoms by moderating the inflammatory responses driven by Aβ deposition, reducing the circulation of pro-inflammatory cytokines, and directly mitigating neuro-inflammation.
- Suppression of oxidative stress – Probiotics may improve AD symptoms by counteracting the oxidative stress.
- Alteration of GIT microbiota composition – Probiotics can increase the growth of Bifidobacteria, while concurrently reducing the growth of opportunistically pathogenic enterobacteria, thereby easing inflammaging and cognitive impairment.
- Changing CNS function via bacteria-derived metabolites – Probiotic bacteria and the fermentation of dietary fibres by the GIT microbiota produce metabolites such as SCFAs (mainly acetate, butyrate, lactate, and propionate), which play a role in the preventative mechanisms of Aβ-induced neuro-inflammation and cognitive impairment. These SCFAs help modulate microglia maturation and function, several signalling pathways, and neurotransmitter synthesis. They may affect the neurotrophic genes including brain-derived neurotrophic factor (BDNF) and nerve growth factor (there is a decrease in BDNF signalling in both the brain and the serum of patients with AD that may be reversed by probiotics).

Most available probiotics products are strains of the Bifidobacterium or Lactobacillus species. Certain Bifidobacterium species are linked to anti-inflammatory properties and reduced intestinal permeability (Vogt *et al.*, 2017). Probiotics may also include bacterial species from other genera such as Streptococcus, Bacillus, and Enterococcus (though there are safety concerns relating to the use of these probiotics, seeing as many pathogenic species are included in these genera) and non-bacterial microorganisms, like yeasts from the genus Saccharomyces. Different probiotic species have various and sometimes strain-specific properties. The effects

of a single probiotic strain should, therefore, not be generalized without separate studies to confirm the effects of other strains. These strain-specific effects, as well as possible side effects and risk factors for taking probiotics, need consideration before patient administration in a clinical setting. Risks include the lack of information for optimal probiotic dosage and frequency, as well as the frequent regulation of probiotics as dietary supplements (instead of biological products or pharmaceuticals), meaning there is generally no obligation to prove safety, potency or purity before marketing probiotic products, leading to possible substantial discrepancies between the indicated and actual contents of these supplements. There are also proposed risk factors for probiotic sepsis, including major risk factors (immunocompromised patients and premature infants) and minor risk factors (central venous catheter, administration of probiotic by jejunostomy, impaired intestinal epithelial barrier, probiotics with high mucosal adhesion properties, concomitant administration of broad spectrum antibiotics to which the probiotic is resistant or probiotics with known pathogenicity). It should be noted that all probiotic bacteremia or fungemia cases were in patients with an underlying immune compromise, a chronic debilitation or disease, and most cases were resolved with suitable antimicrobial therapy (Boyle *et al*., 2006).

1.2.2. Prebiotics

Prebiotics are dietary ingredients that reinforce beneficial GIT microbes (Vulevic *et al.*, 2015) by nourishing and selectively stimulating the growth and/or inhibition of certain colonic bacteria (Toribio-Mateas, 2018). Non-digestible carbohydrates, such as oligosaccharides, act as prebiotics for probiotics and the natural GIT microbes. They become fermented in the lower colon – converting to SCFAs that will nourish the beneficial gut microbes. Oligosaccharides are largely obtained from foods like bananas, onion, garlic, barley, chicory, lentils, honey, sugarcane juice, tomato, milk, mustards, rye, soybeans and wheat (Swapna *et al.,* 2017), as well as culinary spices like cayenne pepper, black pepper, cinnamon, ginger, rosemary, oregano and turmeric. Another example of prebiotics is proanthocyanidins (a flavonoid), which is found in grapes, apples, blueberries, cranberries, bilberries, black currants, cocoa, red wine, pecans, hazelnuts and pistachios (Toribio-Mateas, 2018).

Ingesting a more varied diversity of vegetables and fruits increases the microbial diversity in the GIT, meaning that dietary prescriptions can be used to manipulate a patient's microbial diversity and abundance (Toribio-Mateas, 2018). Prebiotics can potentially undo the age-related decrease in Bifidobacteria and moderate related health factors. Prebiotic dietary interventions may be important in elderly patients for improvement of both their microbial and immune systems (Vulevic *et al.*, 2015). The recommendation of consuming a variety of brightly-coloured fresh foods on a daily basis allows for the ingestion of prebiotics (also a good source of fibre) to provide useful nutrients for neuroprotection. Cruciferous vegetables (e.g., broccoli, cauliflower, cabbage), dark and green leafy vegetables (e.g., chard, kale) and bulbs (e.g., onions, spring onions, garlic, leeks) are among the foods that provide high amounts of fibre as well as nutrients, such as folate and sulforaphane, known for their neuroprotective activity (Toribio-Mateas, 2018).

1.2.3. Synbiotics

Synbiotics are products containing a mixture of probiotics and prebiotics, wherein the prebiotic component selectively favours the probiotic component (Schrezenmeir & De Vrese, 2001). The probiotics are used in combination with specific prebiotics (e.g., a fructooligosaccharide with Bifidobacteria or lactitol with Lactobacilli) (Collins & Gibson, 1999). The health benefits of synbiotic ingestion include the improvement of gut microbiota homeostasis, improved liver function in cirrhotic patients, prevention of bacterial translocation and improved immunomodulation (Pandey, Naik & Vakil, 2015).

Synbiotics were developed to improve the survival of the bacteria passing through the upper GIT (Scholz-Ahrens *et al*., 2007). Possible survival difficulties for probiotics include factors like pH, the presence of organic acids, moisture and oxygen, which may disturb the viability of probiotics – especially dairy products (Pandey, Naik & Vakil, 2015). The prebiotics provide a substrate, e.g. non-digestible carbohydrates, for the synbiotic mixture, which is readily available for sequential fermentation (Collins & Gibson, 1999), consequently over a more expansive range of

the large intestine (Scholz-Ahrens *et al*., 2007). This may improve the viability of the probiotic (Collins & Gibson, 1999), thereby increasing the effects in the large intestine (Scholz-Ahrens *et al*., 2007). The selective activation of growth and metabolism of beneficial microbes and their improved implantation into the colon contribute to maintenance of the intestinal homeostasis (Pandey, Naik & Vakil, 2015).

Synbiotic formulations include probiotics like Lacbobacilli, Bifidobacteria, *S. boulardii* and *B. coagulans*. The frequently used prebiotics include oligosaccharides (fructo-, galacto- and xylose-oligosaccharides), inulin, and natural prebiotics (e.g. chicory and yacon roots) (Pandey, Naik & Vakil, 2015). The combined effect of synbiotics can have an additive (Scholz-Ahrens *et al*., 2007) or synergistic action (Swapna *et al.,* 2017).

1.2.4. Fermented foods

Fermented foods are edible products prepared from cooked or raw animal or plant materials by the addition of microorganisms (either spontaneously or by the addition of starter cultures). Globally, people have learnt to culture and use essential microorganisms (generally lactic acid bacteria, filamentous moulds and yeasts) for the production of fermented foods (primarily as a method of preservation) and alcoholic beverages (Tamang & Fleet, 2009). Carbohydrate fermentation into organic acids and alcohols by microbial enzymes slows the tempo of decomposition – thereby expanding the storage period of perishable foods (Swapna *et al.,* 2017). Fermented foods are also tools that help modulate human GIT microbiota and brain health through the multitude of pathways that connect the GIT and CNS (Toribio-Mateas, 2018).

Among the vital functional microorganisms, yeasts are found in several fermented foods. They are used commercially in the production of products like bread, baker's yeast, cheese, wine and beer (Tamang & Fleet, 2009). Almost 60 species of Lactobacillus (including *L. acidophilus*, *L. bulgaricus*, *L. gasseri, L. casei*, *L. reuteri, L. johnsonii*, *L. plantarum*, *L. rhamnosus* and *L. salivarius)* and Bifidobacterium can be found in fermented foods, including yoghurt, aged cheese, kimchi, kefir and tosa (Swapna *et al*., 2017). Kefir is an intricate combination of bacteria and yeasts or

scoby (a symbiotic culture of yeast and bacteria) and it contains a numerous bacterial species other than Lactobacilli and Bifidobacteria. *Lactobacillus paracasei, L. acidophilus, L. bulgaricus, L. plantarum* and *L. kefiranofaciens* are the predominant species, with *Acetobacter aceti, Lactobacillus kefiri, Acetobacter rasens* and 23 different yeast species (primarily *Saccharomyces cerevisiae, Saccharomyces unisporus, Candida kefyr* and *Kluyveromyces marxianus*) also having been isolated in milk kefir. Daily consumption of kefir increases secretory IgA in faeces, while decreasing pro-inflammatory cytokines expression in the GIT (Toribio-Mateas, 2018). In Asia, moulds, and to some extent yeasts, are predominantly used in the fermentation processes. In the rest of the world fermented products are made with bacteria exclusively or bacteria-yeast mixed cultures (Tamang & Fleet, 2009).

Fermented food ingestion may result in mental health improvements similar to supplementation with a probiotic capsules. Their comparably effective results support the ingestion of live probiotic bacteria from food products rather than the mandatory ingestion of probiotic supplements, with the additional advantages of lower cost and improved patient acquiescence. Other than the advertised health benefits, some people find a greater appeal in eating these “functional foods” than taking probiotic supplements (Toribio-Mateas, 2018).

1.3. Measurements of cognitive function

The most frequent cases of periodic memory lapses are in Mild Cognitive Impairment (MCI) patients who later progress to an AD diagnosis (Albert *et al*., 2011). An AD diagnosis can currently only be ascertained with an autopsy (Perrin *et al*., 2009). The deficiency of routine diagnostic tools for early detection of AD is an aggravating factor in this growing medical predicament (DeTure & Dickson, 2019).

Various memory tests are available (for identifying patients who will likely develop AD) and are especially useful in settings where cognitive symptoms are subtle or secondary mental illnesses are present (Perrin *et al*., 2009). Alzheimer’s can compromise memory as well as other cognitive domains, which therefore also need examination (Albert *et al*., 2011). Cognition tests can gauge the various mental domains (including memory, attention, concentration, orientation, intellect, language,

executive function, visuospatial abilities and mood) and repeat tests can track the progress of an individual (Perrin *et al*., 2009). These tests include the Rey Auditory Verbal Learning Test, the California Verbal Learning Test and the Wechsler Memory Scale. Tests are also available to assess selective cognitive domains, e.g. the Trail Making Test (executive function), the Boston Naming Test (language), figure copying (spatial skills) and forward digit span test (attention) (Albert *et al*., 2011).

Many characteristic biomarkers of AD are obtainable via structural MRI, molecular PET neuroimaging, and CSF analyses (Dubois *et al.,* 2007*)*. Combined with clinical evaluation and cognitive testing, these biomarkers can contribute to the diagnostic criteria for AD and assist with the differential diagnosis and prognosis of AD (Perrin *et al*., 2009). The use of biomarkers may help to determine the underlying etiology of the disease, the probability of progression in disease severity (Albert *et al*., 2011) and, in some cases, can be precursors of the emergence of cognitive impairment. The biochemical changes in the CNS can be directly studied by sampling plasma and CSF (Perrin *et al*., 2009). Biomarkers in the CSF can detect molecular features that directly reflect the pathology of AD. They offer proof of the presence of key proteins (like Aβ and tau) deposited in the brain during the progression of the disease (Albert *et al*., 2011). Due to oxidative harm also being implicated as a mediator of AD (Perrin *et al.,* 2009), there are indirect or nonspecific biomarkers of neuronal injury also present to provide evidence of AD (Albert *et al*., 2011). Increased levels of lipid peroxidation by-product (isoprostanes) and RNA oxidation (8-hydroxyguanine) in CSF may predict the manifestation of MCI and AD (Perrin *et al*., 2009). The location specific pattern of irregularities may lend these biomarkers specificity for AD (Albert *et al*., 2011).

1.3.1. Cognitive screening tools

1.3.1.1. Mini-Mental State Exam (MMSE)

The MMSE is the near universally recognized screening test for cognitive impairment. It is mostly useful for detecting dementia, but low scores may also be caused by conditions like delirium, depression and non-memory impairments (such

as visuospatial impairments). The MMSE is a brief test (5-15 minutes) during which assessors questions participants regarding orientation, attention, calculation, and recall. Each question is scored and a score of 23/30 is recommended as the cut-off score for dementia, but the predictive validity of the MMSE as a screening tool for dementia depends on educational level, age and cultural background. A score below 24 should be further evaluated (including patient/participant history and more detailed cognitive assessment). For participants with secondary education a score below 26 should be similarly further evaluated, whereas a score of 20 may be normal for elderly people with limited schooling. The MMSE was not designed to monitor change, but a score change of five points is likely to be clinically significant (Roselli *et al.*, 2009).

1.3.1.2. Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)

The RBANS is a brief, individually administered and repeatable tool for measuring cognitive function. This includes testing attention, language, visuospatial abilities, delayed memory and immediate memory (Duff *et al*., 2010). This tool was initially developed for identifying and characterizing abnormal cognitive decline in the elderly and as a neuropsychological screening test for younger patients (Randolph *et al.,* 1998), but has suitable sensitivity in the detection of cognitive impairments associated with numerous neuropsychiatric conditions, including AD, MCI and Huntington's disease (HD). It is a 30 minute test consisting of 12 subtests, which yield five Index scores (scored for five cognitive domains) and a Total Scale score (Duff *et al*., 2010). Although overall RBANS scores may be identical for different conditions, the profiles can differ significantly on the subsections, e.g. AD patients show the poorest performance on Delayed Memory and Language subsections, while the HD patients achieve their lowest scaled scores on the Visuospatial and Attention subsections. These results are in accordance with the neuropsychological profiles of these dementing illnesses (Randolph *et al.,* 1998).

1.3.1.3. Test Your Memory (TYM)

This is an AD detection tool consisting of 10 cognitive tasks filled in by a patient under minimal supervision with a total score of 50 (Agahi *et al*., 2018) covering domains that include orientation, recall, arithmetic, naming and visuospatial tasks. How much help the patient needs is used as the eleventh task of the test. The tool is useful for detecting different dementia types (Brown *et al.,* 2019) and patients with AD score significantly lower on the TYM test than patients with subjective memory complaints (cut off score = 42) (Agahi *et al*., 2018). The TYM detects 80% of atypical Alzheimer's (Brown *et al*., 2019), with a score of <25 indicating severe AD (Agahi *et al*., 2018). Repeat testing shows that AD patients' scores decline at an average rate of 4.1 points annually, while the patients with subjective memory complaints generally show some improvement. The test takes a minimal amount of medical time to administer and administrators need minimal training to oversee and score the test (Brown *et al.,* 2019).

1.3.1.4. Consortium to Establish a Registry for Alzheimer's Disease (CERAD)

The CERAD was aimed at developing standardized, validated measures of cognitive assessment that would be minimally time-consuming, easy to administer by health care personnel, and have high sensitivity and specificity to AD. It is a brief and dependable method of evaluating the presence and degree of cognitive impairment, with an emphasis on repeated evaluation in order to examine AD progression. The CERAD battery has amassed a large database of clinical information for studying the progression of AD (Rossetti *et al*., 2010). The CERAD test battery includes measures in the areas where AD associated impairments first occur (verbal memory and naming). It has a high re-test reliability and longitudinal validity. None of the CERAD memory measures can effectively stage the severity of dementia past the mild stage (Karrasch *et al.*, 2005).

The English-American version of CERAD clinical and neuropsychological assessment batteries was translated to Korean, with the basic structures of all

measures in the original CERAD batteries being maintained in translation (Lee *et al.,* 2002). The CERAD-K includes 11 tests measuring areas of language function, memory function, visuospatial function, attention and executive function (Kim *et al*., 2020).

1.3.1.5. Cambridge Neuropsychological Test Automated Battery (CANTAB)

The CANTAB has been used as to assess cognitive functions in various disorders, e.g. dementia, schizophrenia and depression. The tests can detect changes in neuropsychological performance on various levels, including working memory, executive function, attention, decision making, processing and reaction time. Tests for AD and MCI include the Motor Screening Task (touching the centre point of flashing crosses on a screen), spatial working memory (assesses ability to retain spatial information and to manipulate remembered items in working memory), reaction time (measures the speed of response to a visual target), pattern recognition memory (a test of visual recognition memory), spatial recognition memory, paired associate learning (PAL), delayed matching to sample (tests visual memory in a delayed recognition memory paradigm), Stockings of Cambridge (assesses executive function) and rapid visual information processing (a visual continuous performance task). Outcome measures include the amount of errors made, memory scores and stages completed (Égerházi *et al*., 2007).

The PAL test evaluates conditional learning of pattern–location associations (Allen *et al*., 2016), in which item pairs are presented during one or more learning trials. The test assesses whether or not items have been connected through memory associations (Karantzoulis *et al*., 2011). The total recall on a PAL test is directly linked to the strength of the association between the paired stimuli. Test performance is sensitive to functional changes in the hippocampus and frontal lobes (Allen *et al*., 2016). A consistent associative learning deficit is demonstrated by elderly patients (Karantzoulis *et al*., 2011).

1.3.1.6. Rey Auditory-Verbal Learning Test (RAVLT)

The RAVLT is a neuropsychological assessment tool used to gage verbal memory skills. The nature and severity of memory dysfunction, specific memory processes within an individual, as well as memory function changes over time can be determined. This tool also allows for retrieval in initial encoding being distinguished from retention of information. The RAVLT structure has been adopted by other memory assessments, including the California Verbal Learning Test (CVLT) and the Wechsler Memory Scale (Bean, 2011).

To conduct the test, an examiner reads 15 nouns aloud (list A) over 5 trials and after each trial, participants are asked to recall as many of the words as possible (in any sequence). The number of words recalled during the five repetitions of free-recall provides a measure of immediate memory span. After a sixth trial, consisting of 15 different words (list B), participants are asked to recall as many words from the second list as possible. They are then immediately asked to again recall the words from list A (trial 7), and then again after a 20-minute delay (trial 8). This delayed recall score (trial 7 and 8) assesses participants' retention skill. Thereafter, participants receive a sheet of 50 words containing the words from both lists and 20 distracter words. They are asked to identify the words from lists A and B and specify which list they came from. This is used to test recognition memory (Best *et al*., 2015).

1.3.1.7. Montreal Cognitive Assessment (MoCA)

The MoCA is a brief cognitive screening tool that was developed to screen patients who present with mild cognitive complaints while generally performing within the normal range on the MMSE (Nasreddine *et al*., 2005). It is a more sensitive tool than the MMSE in detecting the presence of MCI and mild AD (90 and 100% sensitivity, respectively), and is used in both research and clinical settings. The MoCA can also measure cognitive impairment in populations independent of Alzheimer's disease, including cases of Parkinson's disease, cardiovascular disease, stroke, and brain metastases (Cooley *et al.,* 2015). In the Japanese version of the MoCA (MoCA-J)

scores range from 0 to 30 (higher scores indicate better cognitive performance) and includes an assessment of the education level (Inoue *et al*., 2018).

1.3.1.8. Wechsler's Adult Intelligence Scale (WAIS) III

The WAIS tests are neuropsychological tools used to measure intelligence, with the WAIS-III being an ensuing revision of the WAIS and the WAIS-R. It provides scores for IQ (verbal IQ and performance IQ), verbal comprehension, working memory, perceptual organization and processing speed. Verbal IQ includes seven tests and provides two of the subindexes – verbal comprehension and working memory (Kaufman & Lichtenberger, 2002). The Verbal Learning Test and story recall are used to evaluate both short- and long-term memories (Chung *et al*., 2014). The working memory index includes an arithmetic and digit span test (selected to measure attention and working memory (Chung *et al.,* 2014)), with letter-number sequencing being used as substitutions for spoiled subtests. Performance IQ includes tests for perceptual organization and processing speed. The perceptual organization index tests include a Block Design test, Matrix Reasoning and Picture Completion test. The processing speed index tests include Digit Symbol-Coding and Symbol Search (Kaufman & Lichtenberger, 2002). During the Digit Symbol-Coding test, participants are required to complete a number of digit symbol substitutes on a printed page as quickly as possible, with the score being the number of squares filled in correctly in 120 seconds (Best *et al*., 2009).

1.3.1.9. CogState brief battery (CBB)

The CBB consists of various tasks including:

- Detection task (playing cards are presented on a computer screen and after random intervals, the card is turned face-up and participants must respond as quickly as possible).
- Identification task (similarly presented as the detection task, but participants are required to respond positively or negatively depending on the colour of the face-up card).

- One card learning task (similar to the identification task, but participants must respond positively if the card has appeared in the previous task and negatively if not).
- One back task (similar to the one card learning task, but participants must respond positively if the card is the same as its immediate precursor and negatively if not).
- Groton maze learning task (requires participants to learn a hidden pathway to trace the path of a target and then repeat the test using the same hidden pathway).
- Social emotional task (requires participants to select the odd picture among a grouping of pictures as quickly and accurately as possible).
- Continuous paired associated learning task.
- International shopping list task (a list of common shopping items are read to the participants, and then subjects are required to recall as many items as possible).

The outcomes are reported as the number of correct or incorrect responses, speed and accuracy of performance (Chong *et al.,* 2019).

1.3.2. Chemical assays

1.3.2.1. 8-hydroxy-2'-deoxyguanosine (8-OHdG)

The activation and maintenance of the degeneration cycle of AD appears to involve the production of ROS (Barbagallo *et al*., 2015). Oxidative DNA damage increases with age and accumulates in the brain, playing a major role in neurocognitive aging and the development of neurodegenerative disease (Zhang *et al*., 2013). The continuous attack of ROS on DNA leads to the hydroxylation of DNA bases (Lovell *et al.*, 1999). The most prominent type of DNA oxidation is 8-OHdG, a nucleic acid modification mainly resulting from hydroxide attack of guanidine (Barbagallo *et al*., 2015), which serves as a common and efficient biomarker of immediate oxidative DNA damage (Lovell *et al*., 1999). Age-dependent increases of 8-OHdG occur in various tissues (e.g. the pituitary gland, liver, kidney, cerebral cortex and cerebellum) (Zhang *et al*., 2013) and have a statistically significant positive correlation with

neuropathologic severity. The base-specific repair of 8-OHdG in damaged DNA cleaves the altered base, then transports it to the CSF and blood, and eventually excretes it in urine. In the case of AD, where 8-OHdG levels in intact DNA are increased (indicating increased oxidative stress) and free repair product levels are decreased (indicating deficient repair mechanisms for removal of oxidized bases) the brain may be subject to dual insult (Lovell *et al*., 1999). Oxidative stress can be assessed by means of an enzyme immunoassay for the measurement of urinary 8-OHdG (Barbagallo *et al*., 2015), which can be used as an index of the average rate of oxidative damage in the whole body, and serve as a pre-screening tool for AD diagnosis (Zhang *et al*., 2013).

1.3.2.2. Brain-derived neurotrophic factor (BDNF)

Levels of BDNF can be compromised from an early stage of MCI/AD (Tanila, 2017). There is a broad expression of BDNF and its receptor in the CNS, where it plays an important role in intracellular signalling, neuronal differentiation, -protection and -survival, synaptic plasticity and brain development (Pláteník *et al*., 2014). A reduction in BDNF signalling causes impaired spatial memory, reduction in neuroplasticity and reduced levels are seen in post-mortem AD brain samples, whereas overexpression of its receptor causes memory improvement and higher BDNF serum levels lead to a slower rate of cognitive decline. Brain-derived neurotrophic factor can be affected by Aβ proteins: directly by the inhibition of pro-BDNF conversion to BDNF and indirectly by Aβ interference with its synaptic transport (Tanila, 2017). Declining serum BDNF levels are some of the most frequent biomarkers of depressive disorder, also implicated in schizophrenia, addiction, eating disorders and may serve as a biomarker for AD diagnosis, prognosis and treatment monitoring (Pláteník *et al*., 2014).

Although there appears to be a negative correlation between age and BDNF concentration in platelet-rich plasma (indicating a decreased natural neurotrophic BDNF action in the elderly and a possible link to age-related neurodegenerative disorders), much controversy can be found on BDNF levels in AD patients, likely due to the stage of the disease. Notably increased BDNF serum levels can be seen in

early stage AD, possibly due to a compensatory increase (Pláteník *et al*., 2014), but higher, lower or unchanged circulating BDNF levels have been seen irrespective of disease severity. The most probable explanation is methodological variation. In a study, the lack of control of confounding factors that influence circulating BDNF levels regardless of dementia (age, gender, depression, medications, lifestyle and experimental issues like the sample storage duration) are likely to bias results and prevent study comparability (Balietti *et al*., 2018). Although there is a lack of consistency in serum BDNF levels in AD, healthy elderly subjects with increased serum BDNF levels do show increased protection against developing AD. Serum BDNF can be of value in predicting the risk of AD, but lacks sufficient power as a singular, isolated biomarker (Tanila, 2017).

2. RESEARCH DESIGN AND METHODOLOGY

2.1. Research Question

Does the empirical evidence regarding the use of probiotics/prebiotics/synbiotics to improve cognitive function in AD/Dementia patients show a combined overall trend to support its routine use?

2.2. Hypotheses

There is a correlation between dysbiosis and cognitive function in AD. The use of selective probiotics, prebiotics and/or synbiotics may alleviate symptoms of AD and improve cognitive function.

2.3. Problem statement

Ideally, diseases related to cognitive decline should be treated before or during the inception of the illness in the human body. Currently the available medications focus on treating symptoms of AD rather than the causative factors of the illness. Seeing as no current cure for AD is available, considering alternative approaches, like the health of the gut-brain axis, may be significant for developing an understanding and an overall improvement in cognitive function.

2.4. Aims and objectives

Manipulation of the GIT microbiota could be investigated as a possible safe addition to the preventive/therapeutic tools in the treatment of AD/dementia. Further research is needed to test how antibiotics/ prebiotics/ probiotics may influence the prevention of AD (Luca *et al*., 2019). The aim of this study is to discover if selective products and foods that strengthen the health of human GIT microbiota (probiotics/ prebiotics/ synbiotics) also improve cognition.

The objectives are to create a pool of data from existing relevant studies and to analyze the pooled results from these various studies to allow for a determination of greater statistical significance than any one of the individual studies.

2.5. Study Design and Methods

A literature review and meta-analysis was conducted – pooling data from various studies already conducted on the subject. Tawfik, *et al.* (2019) provides a step by step guide for conducting a systematic review and meta-analysis (Figure 1).

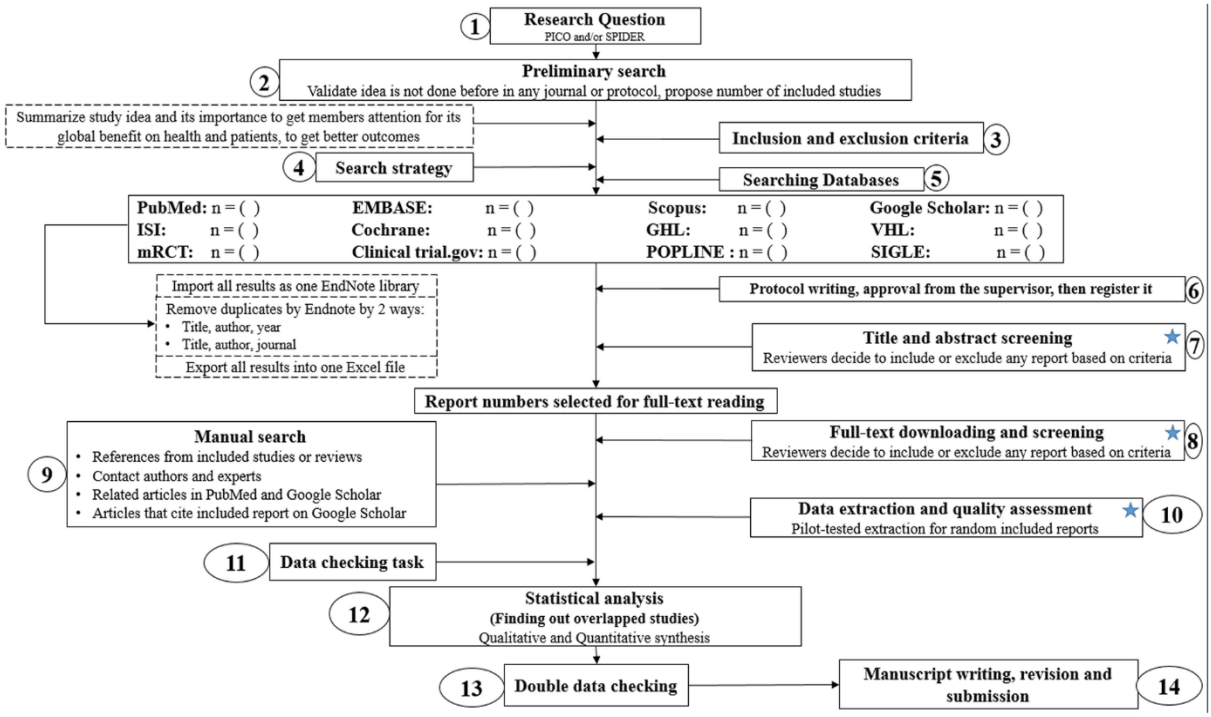

Figure 1: A step by step guide for conducting a systemic review and meta-analysis (From Tawfik, *et al*., 2019).

The first step in the procedure of this study, after approval of the protocol, was to complete a literature search according to predetermined inclusion and exclusion criteria. Secondly, the data recorded in the selected studies (in this case recorded as continuous data) was extracted and entered into the Review Manager 5.3 (RevMan) program for analysis. RevMan is freely downloadable software developed by The Cochrane Collaboration (mostly for use with Cochrane reviews). RevMan

provides guidelines and tools for all aspects of a systematic review, including meta-analysis (Borenstein *et al*., 2009).

According to Higgins & Green (2011), various options for data analysis of continuous data exists and should be considered before an analysis is performed and interpreted, depending on the data extracted from the selected studies:

- Firstly, either the inverse-variance fixed-effect method or the inverse-variance random-effects method of analysis should be chosen. If there is no heterogeneity among the selected studies for the analysis, the methods will yield exactly the same results. For heterogeneous studies, when using the random-effects method, the overall intervention effect confidence intervals will be wider and matching p-values will be less significant compared to the fixed-effect method.

- Secondly, one of two summary statistics, the mean difference (MD) or the standardized mean difference (SMD) should be selected for the data analysis, depending on whether studies all report the outcome using the same scale (MD) or using different methods of measurement associated with different scales (SMD). The MD estimates the average change in outcome between the intervention group compared to the control group. When performing a meta-analysis using this summary statistic for each study, the standard deviations of the study observations and sample sizes are used to calculate the weight assigned to each study. When the methods of measuring the same outcome in selected studies for analysis differ, the SMD is used to standardize the studies' results to the same scale before combining them. For each study, the SMD expresses the intervention effect size relative to the observed variability – if the difference in means is the same proportion of the standard deviation in two studies, they will have the same SMD, irrespective of their actual methods of measurement. The danger of the SMD method is that it assumes that differences in standard deviations among studies reflect differences in measurement scales only and not true differences in variability among study populations.

- Thirdly, a selection should be made between an analysis based on change from baseline values or final (post-intervention) values of outcome measures. An analysis based on change values may be more efficient and powerful than comparison of final values in some cases, as it eliminates some of the between-person variability, but it may be practically less efficient for outcomes which are unstable or difficult to measure precisely, leading to the measurement error being larger than the actual between-person baseline variability.

The studies included in this review made use of participants in different age groups and different medical conditions. The outcome measures of the studies included in this review are different – hence a SMD random effects model is considered to be more appropriate for analysis of the data. Final values of outcome measures were used, considering that change means and standard deviations thereof were not available for all the data sets. Finally, the effect size associated with the pooled data on the overall and sub-analyses, along with data regarding heterogeneity of the analysed studies were used to draw conclusions.

2.5.1. Data collection

Using online access, a preliminary search for studies was done on Google Scholar, Cochrane Library and PubMed. A search was done on the terms ‘gut brain axis’, ‘brain AND microbiome’, ‘dysbiosis AND probiotics/ prebiotics/ synbiotics’, ‘dysbiosis AND Alzheimer’s’, ‘probiotics/ prebiotics/ synbiotics AND Alzheimer’s’, ‘probiotics/ prebiotics/ synbiotics AND cognitive function’ and ‘dysbiosis AND cognitive function’. Related links were followed to secondary sites. Secondly the data was siphoned according to the PRISMA guidelines (Figure 2). This included:

1. Removing duplicate records from the collected articles.
2. Examining titles and abstracts among the collected articles and removing those that were plainly irrelevant.
3. Examining the full text of potential articles for inclusion and determining if they met eligibility criteria.

4. In cases of incomplete reporting – contacting the authors for raw data and/or clarification on the articles.
5. Collecting data from the final selection of articles.

The inclusion criteria for studies were according to the following measures:

1. Studies needed to be controlled trials (randomized controlled trials, case series or crossover studies), with human participants, where mental faculties were measured according to a valid test at least twice during the study – pre- and post-intervention. Randomized controlled trials were preferred, due to lower risk of bias.

2. Studies where the participants had a relevant Alzheimer's/Dementia diagnosis were preferable, but considering Alzheimer's pathology could start as early as puberty or young adulthood (Braak & Del Tredici, 2010) studies measuring cognition and memory in human participants without a diagnosis were also included.

3. All studies needed to be fully available in English and available as pdf downloads.

Exclusion criteria included:

1. Any study that was an abstract only.

2. Was published before 2005.

3. Had incomplete data in terms of participant follow-up.

4. Studies that had only observational data and no quantifiable measure of cognitive function.

5. Studies that were determined to be non-relevant to this study.

Studies were individually screened according to abstracts and relevant studies were then fully read to determine suitability for inclusion. Figure 2 shows the step by step search, selection and inclusion of published articles that were included in this study.

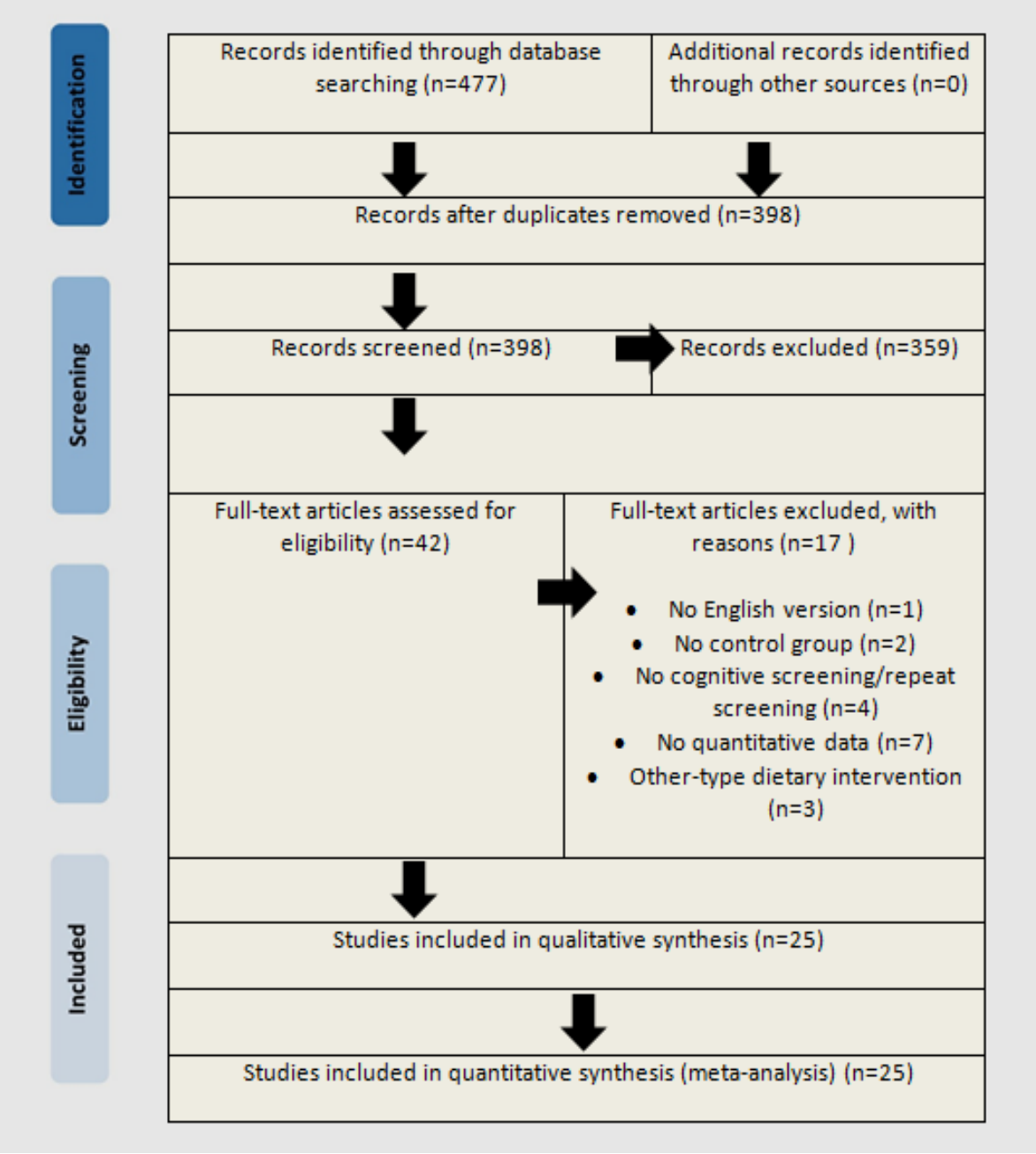

Figure 2: PRISMA flow diagram of literature search (created using RevMan).

2.5.2. Elimination of bias.

In order to reduce the risk of bias, a co-reviewer was used to assist in the steps of individual screening and selection of suitable studies. Both reviewers assessed the titles and abstracts of studies found on the search engines before determining which studies were irrelevant and which ones needed further screening. Full study screening was done independently. The assessment was done without blinding of the authors or journal of publications, but results were disregarded past the point of determining that quantifiable result data was provided. Studies were only included once both reviewers reached consensus. Seventeen studies were immediately agreed upon, eight more were included after deliberation. Once suitable studies were collected, relevant data from each study was extracted (Table 1).

With regards to the studies included in the analysis, the risk of bias in the results of each of the studies was considered. The classification used for detection of bias was: selection bias (determining sequence generation and allocation concealment), performance bias (determining blinding of participants and personnel), attrition bias (determining completeness of outcome data), detection bias (determining blinding of outcome assessment) and reporting bias (differences between reported and unreported findings) (Higgins & Green, 2011). In Figure 3 the overall bias of the selected studies, according to author review, can be seen with green representing low risk, white an unclear risk and red a high risk of bias. None of the included studies were free of bias risk, but the overall risk of bias was low.

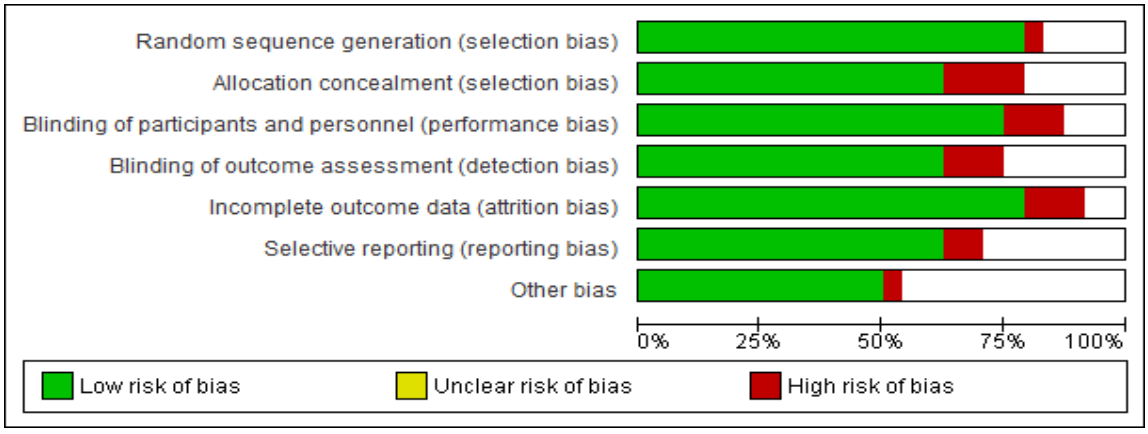

Figure 3: Risk of bias analysis for included studies (created using RevMan).

2.5.3. Data captured

Data to be extracted included: demographic data of participants, intervention, dose and duration of treatment for experimental and control groups, outcome measures and sample size.

The summary statistics required for each test outcome are determined by the type of outcome data (Higgins & Green, 2011). To perform a meta-analysis of continuous outcome data the summary statistics to be included were:

- The mean value of the outcome measurements for the experimental and control group respectively.
- The standard deviation or variance of the outcome measurements for the experimental and control group respectively.
- The number of participants in each intervention group.

Whenever any of the above summary statistics were not available directly, alternative statistics were used to calculate or estimate the missing value. For example, in the case of a missing standard deviation, a standard error, a p-value, a confidence interval, an observed t-statistic or F-statistic value were used to calculate the standard deviation (Higgins & Green, 2011).

2.5.4. Outcome measures

The main outcome measure for this review was the standardized mean difference (estimated effect size). For each of the selected studies the standardized mean difference between the probiotic/ prebiotic/ synbiotic treatment groups and the placebo groups had to be determined.

2.5.5. Data analysis

Of the data extracted from the studies to be processed through RevMan, not all results were in the same format. Some studies reported a mean, SEM (standard error of mean) and sample size while other studies reported the mean and

confidence interval. In order to calculate the effect sizes, the data formats from the different studies needed to be homogenized. All of the data was converted to the mean and standard deviation (SD) of the outcome measures, for both the experimental and placebo groups (Table 2). In the studies where results were expressed as mean ± SEM, the standard error of the mean was converted to standard deviation of the observation using equation 1 (Higgins & Green, 2011).

$$SD = SE \times \sqrt{n} \qquad \text{- equation 1}$$

Where *n* = number of observations.

In cases where a decreased score indicates improvement in cognition (e.g. a decreased score in mental flexibility (Kim *et al.,* 2020) or a decreased 8-OHdG score (Barbagallo *et al*., 2015)), the mean values were multiplied by -1 in order to correct the direction of the scale, while the standard deviations were left unchanged (Higgins & Green, 2011).

Some studies provided a singular score for cognition, which was directly extracted and used, whereas other studies provided scores according to various neurocognitive function tests (e.g. PAL, Working Memory, and Processing Speed). In the case of the latter, if a composite score was provided, it was used directly. Otherwise, if a composite score was not provided, the composite mean of the various tests was calculated using equation 2 (Borenstein *et al*., 2009).

$$Y = \frac{1}{m}\left(\sum_{j}^{m} Y_j\right) \qquad \text{- equation 2}$$

where Y denotes the combined mean effect across the outcomes within a study, m denotes the number of tests performed/measured within a study and Y_j denotes the outcome of the j^{th} test.

The variance of the composite mean was calculated using equation 3 (Borenstein *et al*., 2009).

$$var\left(\frac{1}{m}\sum_{i=1}^{m} Y_i\right) = \left(\frac{1}{m}\right)^2\left(\sum_{i=1}^{m} V_i + \sum_{i\neq j}\left(r_{ij}\sqrt{V_i}\sqrt{V_j}\right)\right) \qquad \text{- equation 3}$$

where V_i denotes the variance of the i^{th} test outcomes and r_{ij} denotes the correlation between the outcomes of the i^{th} test and the outcomes of the j^{th} test.

For studies for which the individual test observations were not provided and hence r_{ij} could not be calculated directly a correlation of 1 was adopted based on the studies included in the analysis that provided a correlation – showing that there was a high correlation between the separate neurocognitive function outcomes (Borenstein *et al*., 2009).

The absolute (post-intervention) measure values and their standard deviations were entered directly into RevMan and the software was used to conduct a random-effect meta-analysis. Thereafter the RevMan program was used to calculate an effect size (Hedges' *g)* and variance for each study, as well as the overall summary effect, confidence intervals and measures of heterogeneity. The RevMan program calculated a summary statistic for the intervention effect of each included study and then a pooled intervention effect estimate using equation 4.

$$\textit{Weighted averages} = \frac{sum\ of\ (estimate \times weight)}{sum\ of\ weights} = \frac{\sum Y1W1}{\sum W1} \quad \text{- equation 4}$$

where Y_i is the estimated intervention effect for the *i*th study and W_i is the weight of the *i*th study (Higgins & Green, 2011).

Lastly, the data was presented as forest plots in order to assist with result interpretation.

3. RESULTS

Table 1 is a summary of the data extracted from the selected studies. It shows the author/s, primary disease being investigated, sample sizes, duration of intervention, the experimental/ placebo treatment and dose used in the study, the outcome measure(s) used, and a brief summary of the outcome.

Table 1: Data extracted from selected studies

Author, year	**Primary Disease**	**Sample**	**Period of inter-vention**	**Treatment and dose used**	**Outcome measure**	**Outcome**
Agahi *et al.*, 2018	AD patients (65–90 years old)	N = 48 Placebo (n = 23) and probiotic (n = 25)	12 weeks	Two capsules, each containing a total dosage of 3×10^9 CFU (including either *Lactobacillus fermentum, Lactobacillus plantarum*, and *Bifidobacterium lactis* or *Lactobacillus acidophilus, Bifidobacterium bifidum*, and *Bifidobacterium longum)* was given to the probiotic group every other day.	TYM	The TYM cognitive test was taken before and after the intervention. The pre- and post-treatment scores in the probiotic group were 14.64±1.71 (mean±SEM) and 17.42±2.42, respectively; the values in the control patients were 14.35±2.27 and 17.47±2.89, respectively. Analysis of variance indicated no difference between the two groups [F(3,114) = 0.29, P<0.82]. The change between the scores achieved at the onset and offset of the trials was 12.86%±8.33 (control group) and −9.35%± 16.83 (probiotic group). The unpaired student t-test indicates no statistical difference between the two groups.
Ahmad *et al.*, 2020	Students 18-30 years old	N = 40 Probiotic tablet (n = 20) and yogurt culture drink/food probiotic group (n = 20) Viewed as 2 separate case series	5 days	The probiotic tablet group received 1 tablet (2 billion CFU/100mg of *L. acidophillus*, *L. salivarius*, *B. bifidum* and *S. thermophillus*) to take after food daily. The food probiotic group received 1 bottle of a yogurt culture drink (10 billion strains of *L. casei)* every day after food.	Human benchmark : number, reaction time, verbal, visual, hearing and typing memory	There was an increase in verbal, visual and number memory test scores, as well as continuous concentration scores in both food and tablet probiotic groups (though statistically insignificant). Within the groups there was an increase in all the scores, suggesting a connection between ingestion of probiotics in either form and both short-term memory and continuous concentration.
Akbari *et al.*, 2016	AD patients	N = 60 Probiotic (n = 30) and placebo (n = 30)	12 weeks	The probiotic group received 200ml/day probiotic milk containing *L. acidophilus*, *L. casei*, *B. bifidum*, and *L. fermentum* (2×10^9 CFU/g for each).	MMSE	The probiotic group showed improvement in MMSE score (+27.90%± 8.07) (mean±SEM) compared to the control group (−5.03%±3.00). The difference in results between the two groups was statistically significant ($P <$ 0.001).

Allen *et al.*, 2016	Healthy volunteers	N = 22 (Crossover trial)	4 weeks	*B. longum* 1714	CANTAB	Compared with baseline results, participants made fewer errors on the PAL test both at post-placebo, (T = 7.5, P<0.05) and post-1714, (T = 6.63, P<0.01), a subtle effect at post-1714 that was greater than the placebo response.
Bajaj *et al.* 2008	Patients with non-alcoholic cirrhosis with Minimal Hepatic Encepha-lopathy	N = 20 Yogurt (n = 14) and placebo (n = 6)	60 days	Subjects received probiotic yogurt (with proven culture stability) or a placebo.	Psychometric Tests include NCT-A, BDT and DST (from WAIS)	There was no learning effect – as seen by similar psychometric performances in the placebo group at baseline and at the end of the trial. NCT-A (Mean±SD) Yogurt end point: 30± 4* No Rx end point: 47±10 BDT Yogurt end point: 41±5* No Rx end point: 28±10 DST Yogurt end point: 69± 8* No Rx end point: 54 ±18 ∗Significant improvement compared to baseline (*P* = 0.003 intention-to-treat, *P* = 0.0004 per-protocol on the paired *t*-test).
Barbagallo *et al.*, 2015	40 patients (age 78.2±1.1 years), 28 AD patients, and 12 controls	N = 28 Test (n = 20) and placebo (n = 8)	6 months	FPP (fermented papaya powder – prepared by fermenting the *Carica papaya*) 4.5 grams/day for the probiotic group.	8-OHdG in the urine	In AD patients FPP significantly decreased 8-OHdG (14.1±1.7 ng/mL to 8.45±1.1 ng/mL, *P*<0.01), with no significant changes in controls, showing a non-significant trend towards an increase (from 12.5 ±1.9 ng/ml to 19.6±4.1 ng/mL, P = NS).
Best *et al.*, 2015	Healthy middle-aged adults (45–60 years)	N = 73	Single dose – 3.5 hours testing session	Daily consumption of 4 g of a proprietary mix of non-starch polysaccharides, a rice flour placebo, or a sucrose control.	RAVLT	There was a significant difference between the group performances on tasks of recognition memory, total ($F(2,68) = 3.28$, $P = 0.004$), list A ($F(2,68) = 3.28$, $P = 0.044$), and list B ($F(2,68) = 4.99$, $P = 0.009$), respectively. There was an insignificant trend for those in the polysaccharide group to recognize more words than those in the placebo group (P = 0.07).
Buigues *et al.*, 2016	Older adults (aged 65 and over)	N = 50 Prebiotic (n = 28) and placebo (n = 22)	13 weeks	Daily intake of prebiotic product (mixture of inulin plus FOS) or placebo (maltodextrin).	MMSE	Placebo Group (mean±SEM) Baseline: 26.1±2.2 Post-treatment: 25.9±2.1 Prebiotic Group Baseline: 26.5±3.1 Post-treatment: 26.4±2.2
Chong *et al.*, 2019	Stressed adults	N = 111 Probiotic (n = 56) and placebo (n = 55) (Aged >30 years n = 29 and n = 23 respectively)	12 weeks	Daily probiotic product (*Lactobacillus plantarum* DR7(1×10^9 CFU) and maltodextrin as excipient) intake or placebo containing only maltodextrin.	CBB	The probiotic improved cognitive and memory functions in normal adults (>30 years old) (*P*<0.05), when compared to either the placebo or young adults (<30 years old) groups.

Chung *et al.*, 2014	Healthy older adults (age 65.00 ± 4.14 years)	N = 36 Placebo (n = 10), 500 mg (n = 10), 1000 mg (n = 7), 2000 mg (n = 9)	12 weeks	*Lactobacillus helveticus*-fermented milk (LHFM) or placebo.	Digit-span test, story recall test, and verbal-learning test (VLT)	Significant improvements were observed on VLT and story recall items at the endpoint compared to baseline. However, no significant between-groups differences were observed.
Hwang *et al.*, 2019	Mild Cognitive Impairment patients	N = 100 Test (n = 50) or placebo (n = 50) Participants who completed the trial N = 45 and N = 47 respectively	12 weeks	*Lactobacillus plantarum* C29-fermented soybean (DW2009) 800mg/day or placebo.	Attention, Working memory and Verbal memory functions	Compared to the placebo group, the DW2009 group showed greater improvements in the combined cognitive functions (z = 2.36, p = 0.02), especially for attention (z = 2.34, p = 0.02). Combined cognitive scores: Placebo (mean±SD) Baseline: 0.01±0.68 Follow-up: 0.25±0.65 DW2009 Baseline: -0.27±0.95 Follow-up: 0.18±0.79
Inoue *et al.*, 2017	Healthy elderly subjects (66-78 years)	N = 38 Probiotic (n = 20) and placebo (n = 18)	12 weeks	*Bifidobacterium* supplementation (1.25×10^{10} CFU each of *B. longum* subsp. *longum* BB536, *B. longum* subsp. *infantis* M-63, *B. breve* M-16V and *B. breve* B-3) or the placebo.	The Japanese version of the MoCA	Post intervention scores showed a significant increase in both groups, while the probiotic group flanker task scores increased more significantly than those of the placebo group (0.35±0.9 vs -0.29±1.1, *P*=0.056) (mean±SD).
Kao *et al.*, 2018	Non-hospital subjects (18-60 years old) with psychosis	N = 49 Prebiotics (n = 24) and placebo (n = 25) (Crossover trial)	24 weeks	Daily ingestion of the prebiotic, B-GOS (galacto-oligosacharide with high selectivity toward Bifidobacteria) or the placebo.	Brief Assessment of Cognition in Schizo-phrenia	There was a significant prebiotic pre- versus post- effect on the executive domains (t = -2.114, p = 0.045, n = 24, d = 0.432). Placebo had no effect. The overall prebiotic effect on the composite T-scores was driven by subtests of executive function. Composite Scores (mean±SEM): Pre-BGOS Pre: 33.83±1.501 Post-BGOS Post: 36.17±1.396 Pre-Placebo: 33.67±1.519 Post-Placebo: 35.27±1.699
Kim *et al.*, 2020	Community -dwelling elderly (>65 years)	N = 53 Placebo (n = 26) and probiotics (n = 27)	12 weeks	Two probiotic capsules after a meal in the morning and evening (a daily total of $1x10^9$ CFU of *B. bifidum* BGN4 and *B. longum* BORI in soybean oil). Placebo: 500 mg of soybean oil only.	CERAD-K	The probiotics group showed greater improvement in the mental flexibility test and stress score than the placebo group ($p<0.05$). Placebo (mean±SD) Week 0: 2.15±0.97 Week 12: 2.52±1.68 Probiotic Week 0: 2.72±1.56 Week 12: 2.08±0.85
Kobayashi *et al.*, 2019	Elderly subjects aged 50-80 years with memory complaints	N = 117 Probiotic (n = 59) and placebo (n = 58)	12 weeks	Two capsules daily of approximately 2.0×10^{10} CFU *B. breve* A1 following meals or placebo.	Japanese version of the RBANS and MMSE	RBANS scores: total scores significantly increased against baseline in both the groups (*B. breve* A1: +5.27, P=0.027; placebo: +4.65, P=0.013). The scores for the language and attention subscales significantly

						increased following the intervention, but no significant change from baseline scores were observed between groups. MMSE: total scores significantly increased in both groups (*B. breve* A1: +1.63, *P*<0.001; placebo: +1.76, *P*<0.001). RBANS B.breve change (mean±SD): 5.27±11.25, P=0.87 Placebo change: 4.65±9.46 MMSE B.breve change: 1.63±1.77 Placebo change: 1.76±1.83 P = 0.62
Leblhuber *et al.*, 2018	AD	N = 20	4 weeks	*Lactobacillus casei W56, L. lactis W19, L. acidophilus W22, Bifidobacterium lactis W52, L. paracasei W20, L. plantarum W62, B. lactis W51, B. bifidum W23* and *L. salivarius W24*.	BDNF	Levels didn't differ before and after supplementation with probiotics. Before: 209±126 (mean±SD) (range = 15.6 - 457 pg/ml). After: 201±121 (range = 30.9 - 476 pg/ml). No significant change was observed with any of the cognitive parameters after probiotics supplementation.
Lew *et al.,* 2018	Stressed adults (mean age of 31.7 ± 11.1 years)	N = 103 Probiotic (n = 52) and placebo (n = 51)	12 weeks	Daily administration of 2 g probiotic *L. plantarum* P8 at a fixed dosage of $2x10^{10}$ CFU/sachet or placebo.	CBB	The probiotic showed insignificant effects on cognition and memory parameters in the overall subject population, due to large variations among subjects. It increased the speed for social emotional cognition (MD = 0.079; 95% CI [-0.128; -0.03]; P = 0.002), attributed to improvement in women while men did not display any differences as compared to the placebo.
Ohsawa *et al.*, 2018	Healthy middle-aged adults with self-identified forgetful-ness or forgetful-ness identified by a close relative or acquaint-tance	N = 60 Placebo (n = 29) and test group (n = 31)	8 weeks	*Lactobacillus helveticus*-fermented milk drink containing Lactononadeca peptide (190 g/day) or the equivalent amount of a placebo drink once a day.	Japanese version of the RBANS test	Statistically significant improvement in the total score, attention score and delayed memory score of participants who received the fermented milk drink was observed. There was significant difference in the attention score between the placebo and test groups post-intervention (p=0.028). Placebo (mean±SD) Before intake: 44.7±5.0 After: 46.5±8.2 Test group Before intake: 44.5±5.1 After: 48.4±8.1
Roman *et al.*, 2018	Fibro-myalgia patients	N = 31 Probiotic (n = 16) and placebo (n = 15)	8 weeks	6 million units per capsule*: L. rhamnosus, LGG L. Casei, L. Acidophilus,* and *B. Bifidus.* Participants took two pills at least 30 min before breakfast and dinner.	MMSE	Probiotic (mean±SEM) Pre-intervention: 28.50±0.42 Post-intervention: 28.44±0.42 Placebo Pre-intervention: 28.47±0.42 Post-intervention: 28.73± 0.28 P = 0.5
Rudzki *et al.*, 2019	Patients with depression	N = 79 *LP299v* (n =	8 weeks	SSRI with the probiotic *LP299v (*containing $10x10^9$	Attention and Perceptivity Test (APT)	There was an improvement in APT and in CVLT total recall of trials 1-5 in the probiotic group

	on SSRI treatment	40) and placebo (n = 39)		CFU *L. plantarum 299v)* or a SSRI with the placebo. All subjects took 1 capsule in the morning and 1 at night.	and the California Verbal Learning Test (CVLT) – correlates with WAIS-R	compared with the placebo. Since patients from both groups did not have any errors in APT those results did not meet the criteria for any statistical analysis.
Sanborn, 2019	Middle-aged and older adults (aged 55-75)	N = 148 LGG (n = 78) and placebo (n = 70)	12 weeks	L.GG blend (10 billion CFUs) including L.GGb, L.GGm, and L.GGe. Placebo: veggie capsule with 250mg purified wood pulp (filler) and a 75mg cellulose cap. All subjects took two capsules daily.	NIH Toolbox for the Assessment of Neurological and Behavioral Function – Cognition	LGG (mean±SD) Base: 49.1±9.87 Post: 52.7±8.48 Placebo Base: 47.0±10.5 Post: 50.4±9.88
Smith *et al.*, 2015		N = 47 (Crossover trial – two sessions a week apart)	Single dose – 4 hours	Oligofructose-enriched inulin (5 g) was given or placebo (maltodextrin).	Immediate Free Recall, Delayed Recall, Logical Reasoning, Semantic Processing and Spatial Memory	The most consistent effects were on the episodic memory tasks where consumption of inulin was related to greater accuracy on the recognition memory task and improved recall performance (immediate and delayed).
Suzuki *et al.*, 2019	Community-dwelling older (aged >70 years) Japanese women with MCI	N = 71 Test group (n = 36) and placebo group (n = 35)	3 months	The test group was provided with 33.4 g MFC (camembert cheese) daily, and the placebo group was provided with the same amount of non-MFC (processed cheese made from mozzarella cheese and cream cheese).	BDNF and MMSE	Significant interactions were observed in BDNF after MFC intake in the secondary (F = 5.368, P = 0.024) and combined analyses (F = 4.354, P = 0.039) but not the primary analysis. There were no significant changes in the MMSE score. MMSE MFC (mean±SD) Baseline: 26.9±2.5 After: 26.9±3.1 MMSE Non-MFC Baseline: 26.6±2.6 After: 27.7±2.1 P=0.022
Tamtaji *et al.*, 2019	Patients with AD	N = 79 Selenium plus probiotic (n = 27), selenium (n = 26) or placebo (n = 26)	12 weeks	Selenium (200 mg/day) plus probiotic containing *L. acidophilus, B. bifidum*, and *B. longum* ($2x10^9$ CFU/day each), selenium (200 mg/day) or placebo.	MMSE	Compared with only selenium and placebo, probiotic and selenium co-supplementation resulted in a significant increase in MMSE score (mean±SD) (+1.5±1.3 vs. +0.5±1.2 and -0.2±1.1, respectively, P<0.001).
Ton *et al.*, 2020	Patients with AD	N = 13 Cognitive assessment was made before (T0) and after 90 days (T90)	90 days	Probiotic-fermented milk (2 ml/kg/daily). Pasteurized milk inoculated with 4% kefir grains containing *A. aceti, Acetobacter sp., L. delbrueckii, L. fermentum, L. fructivorans, E. faecium, Leuconostoc spp., L. kefiranofaciens, Candida famata*, and *C. krusei.*	MMSE	Most subjects exhibit a clear improvement in memory, visuospatial, and executive/ language functions. An improvement of performance in MMSE in 28% (T0: 17:4±1:03 and T90: 22:3±0:82, p<0.0001) (mean±SD) was observed, indicating the benefits of kefir-fermented milk on cognitive status.

Table 2 lists the converted data values from the data in Table 1 to the mean and standard deviation (SD) of the outcome measures, for both the experimental and placebo groups. In the studies where results were expressed as mean ± SEM, the standard error of the mean was converted to standard deviation of the observation using equation 1 (Higgins & Green, 2011).

Table 2: Converted/Extracted overall cognitive measures

Study ID	**Experimental**				**Control**			
	Initial score (Mean:SD)	**End Score (Mean;SD)**	**Change score (Mean: SD)**	**Total (n=)**	**Initial score (Mean;SD)**	**End score (Mean;SD)**	**Change Score (Mean;SD)**	**Total (n=)**
Agahi *et al.*, 2018	14.64; 8.55	17.42; 12.1	2.78	25	14.35; 10.8865	17.47; 13.86	3.12	23
Ahmad *et al.*, 2020	26.8475; 9.575	31.4225; 11.05	4.575	20	30.7475; 9.9075	33.1675; 9.5631	2.42	20
Akbari *et al.*, 2016	8.67; 7.8872	10.57; 8.9827	1.9	30	8.47; 6.0249	8; 5.9154	-0.47	30
Allen *et al.*, 2016	-3.795; 3.0178	-1.5365; 2.0561	2.2585	22	-3.795; 3.0178	-2.705; 3.1884	1.09	22
Bajaj *et al.*, 2008	48; 5.1995	55; 6.412	7	14	36.5; 10.7915	41; 13.71	4.5	6
Barbagallo *et al.*, 2015	14.1; 1.7	8.45; 4.91	-5.65	20	12.5; 1.9	19.6; 11.59	7.1	8
Best *et al.*, 2015	-	-	-1.13; 5.8	23	-	-	-1.58; 6.374	26
Buigues *et al.*, 2016	26.5; 16.4037	26.4; 11.6413	-0.1	28	26.1; 10.3189	25.9; 9.8499	-0.2	22
Chong *et al.*, 2019	-	98.058; 20.334	-	29	-	96.434; 15.5	-	23
Chung *et al.*, 2014	10.083; 11.481	7.459; 11.363	-2.624	25	7.567; 10.343	9.433; 12.552	1.866	10
Hwang *et al.*, 2019	-0.27; 0.95	0.18; 0.79	0.45	50	0.01; 0.68	0.25; 0.65	0.24	50
Inoue *et al.*, 2017	23.5; 3.4	25.8; 3.6	0.35; 0.9	20	23.9; 2.9	25.8; 2.5	0.29; 1.1	18
Kao *et al.*, 2018	33.83; 7.353	36.17; 6.839	2.34	24	33.67; 7.595	35.27; 8.495	1.6	25
Kim *et al.*, 2020	-	-	3.68; 2.69	27	-	-	-3.32; 2.35	26
Kobayashi *et al.*, 2019	26.1; 0.96	27.73; 1.62	1.63; 1.77	59	26.31; 0.9	28.07; 1.62	1.76; 1.83	58
Leblhuber *et al.*, 2018	209; 1226	201; 121	8	20	-	-	-	-
Lew *et al.,* 2018	-	6.57; 1.1494	-	52	-	6.63; 0.8889	-	51
Ohsawa *et*	44.5; 5.1	48.4; 8.1	3.9	31	44.7; 5	46.5; 8.2	1.8	29

al., 2018								
Roman *et al.*, 2018	28.5; 1.68	28.44; 1.68	-0.06	16	28.47; 1.6267	28.73; 1.0844	0.26	15
Rudzki *et al.*, 2019	367.305; 54.219	421.365; 59.8841	54.06	30	382.7; 664.324	398.63; 57.403	15.93	30
Sanborn, 2019	49.1; 9.87	52.7; 8.48	3.6	78	47; 10.5	50.4; 9.88	3.4	70
Smith *et al.*, 2015	-	79.2; 7.438	-	47	-	77.6; 6.9149	-	47
Suzuki *et al.*, 2019	26.9; 2.5	26.9; 3.1	0.1	36	26.6; 2.6	27.7; 2.1	0.9	35
Tamtaji *et al.*, 2019	9.4; 3.5	10.9; 3.8	1.5; 1.3	27	9.3; 4.1	9.1; 4.4	-0.2; 1.1	26
Ton *et al.*, 2020	17.4; 1.03	22.3; 0.82	4.9	13	-	-	-	-

3.1 RevMan forest plots

Each figure (Figures 4-12) indicates the weight, effect estimate and 95% confidence interval of the effect estimate for the individually included studies as well as the total combined effect. Figures 7-12 show the pooled scores for analyses of separate cognitive tasks as indicated in the various studies, with each forest plot including only the studies where the separate cognitive function/task was measured. Each study shows a correlating line on the forest plot, with the size of the block indicating the weight assigned to the study and the line indicating the 95% confidence interval. The larger the weight of a study is, the bigger the contribution to the calculation of the pooled result.

3.1.1. Cognitive ability

Figure 4 shows the pooled results for 755 experimental and 697 control participants.

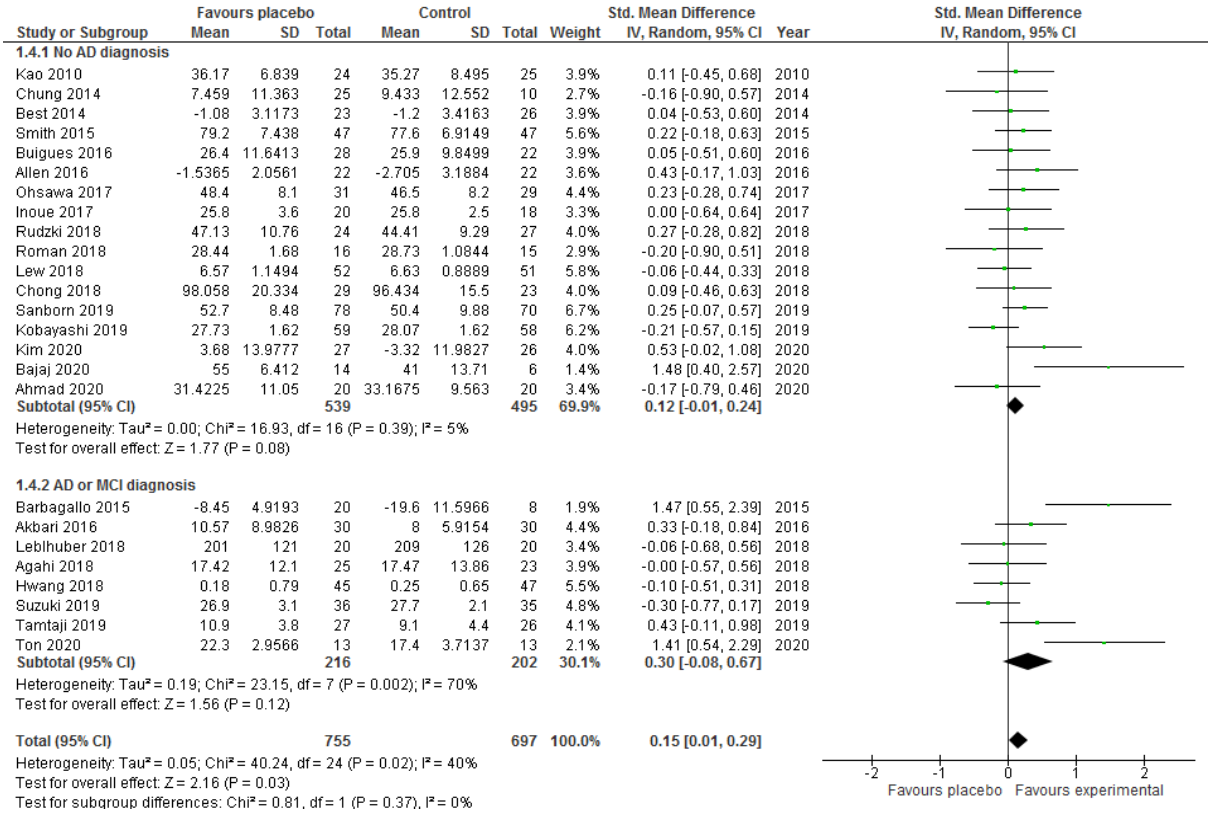

Figure 4: Total cognition score forest plot extracted from RevMan.

The effect estimate for cases with no AD diagnosis is 0.12 with a 95% CI of [-0.01; 0.24] (z=1.77, p=0.08) and heterogeneity scores of the included studies of I^2=5% for a total of 539 experimental and 495 control participants. For the cases where an AD/MCI diagnosis was made, the overall effect estimate is 0.3 with a 95% CI of [-0.08; 0.67] (z=1.56, p=0.12) and heterogeneity scores of the included studies of I^2=70% and p=0.002 of Chi^2-value for a total of 216 experimental and 202 control participants. The total effect estimate is 0.15 with a 95% CI of [0.01; 0.29] (z=2.16, p=0.03) and heterogeneity scores of the included study results of I^2=40% and p=0.02 of Chi^2. Two subgroups were created to show the pooled effects and heterogeneity of studies where a diagnosis for AD or MCI was made compared to the studies where a diagnosis for these conditions were not made.

3.1.1.1. Cognition of MCI or AD diagnosed patients

Figure 5 shows an analysis of the studies where a diagnosis of MCI or AD was made among the participants, according to the supplements used.

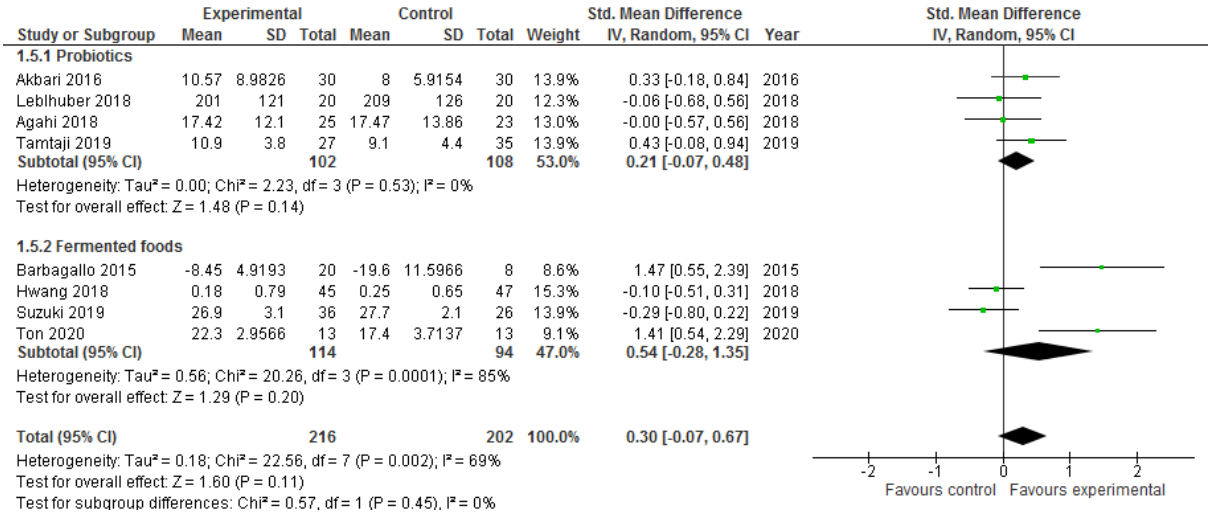

Figure 5: Total Cognition of MCI or AD diagnosed participants – sub-analysis according to supplements (extracted from Revman).

Subgroups of the analysis shows the effect estimates of 0.21 (95%CI [-0.07; 0.48], p = 0.14, z = 1.48) for studies where a probiotic was used and 0.54 (95%CI [-0.28; 1.35], p = 0.2, z = 1.29) for studies where fermented foods were used. The overall effect estimate is 0.3 (95%CI [-0.07; 0.67], p = 0.11, z = 1.6) with heterogeneity of I^2 = 69% and p = 0.002 for the Chi^2-value.

3.1.1.2. Cognition sub-analysis according to supplement

Figure 6 shows the total cognition with sub-groups according to the type of supplement that was used for the experimental group of each study.

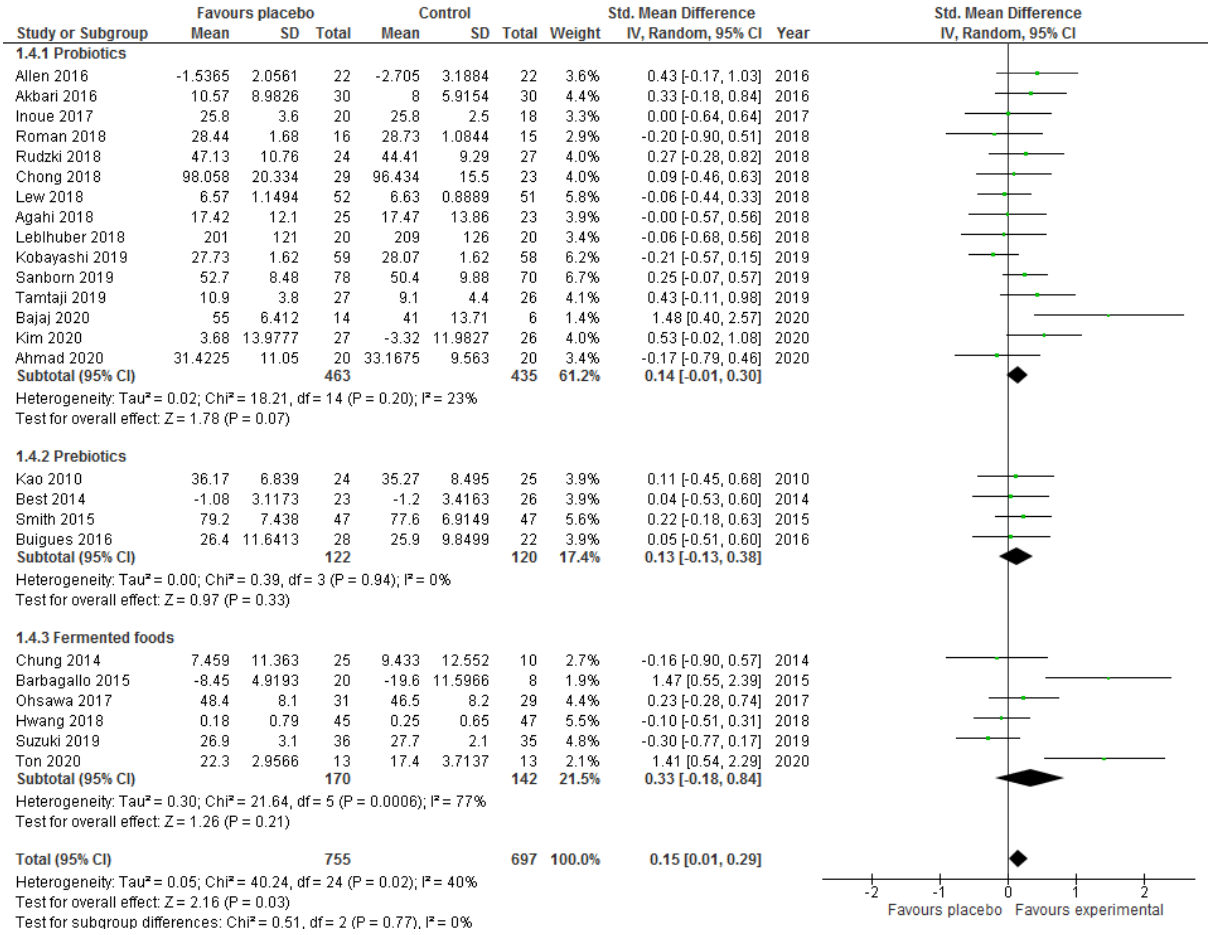

Figure 6: Total Cognition sub-analysis according to supplement (extracted from Revman).

Synbiotic supplementation was included under 'Probiotics'. The probiotics group shows an estimated effect of 0.14 (95%CI [-0.01; 0.3], z = 1.78, p = 0.07) with heterogeneity I^2 = 23% and p = 0.02 for the Chi^2-value for 463 experimental and 435 control participants. The prebiotics group shows an estimated effect of 0.13 (95%CI [-0.13; 0.38], z = 0.97, p = 0.33) with heterogeneity I^2 = 0% and p = 0.94 for the Chi^2-value for 122 experimental and 120 control participants. The fermented foods group shows an estimated effect of 0.33 (95%CI [-0.18; 0.84], z = 1.26, p = 0.21) with heterogeneity of I^2 = 77% and p = 0.0006 for the Chi^2-value for 170 experimental and 142 control participants.

3.1.2. Paired Associate Learning (PAL)

Figure 7 shows the pooled results for the PAL test for a total of 103 experimental and 96 control participants.

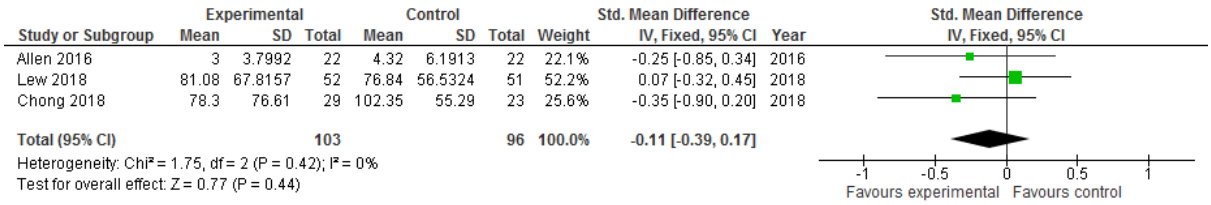

Figure 7: Paired Associate Learning test forest plot extracted from RevMan.

The overall effect estimate is -0.11 with a 95%CI of [-0.39; 0.7] (z=0.77, p=0.44). The heterogeneity of the study results is I^2=0%.

3.1.3. Processing speed

Figure 8 shows the pooled results of cognitive processing speed for 251experimental and 239 control participants.

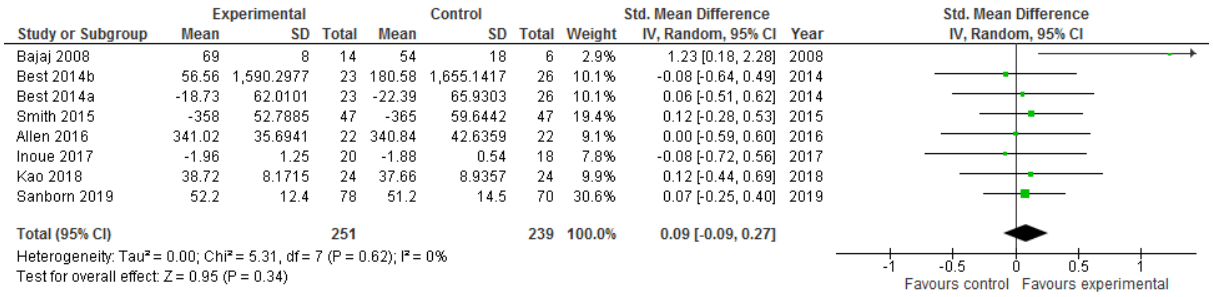

Figure 8: Processing speed forest plot extracted from RevMan.

The overall effect estimate is 0.09, 95%CI of [-0.09; 0.27] (z=0.95, p=0.34) and heterogeneity of I^2=0% and p=0.62 for the Chi^2-value.

3.1.4. Short term/Working memory

Figure 9 shows a combined effect estimate of 0.17 (95%CI [0.04; 0.30], z=2.52, p=0.01) for short term or working memory scores of 586 experimental and 546

control participants. Heterogeneity scores for the study data show I^2=0% and p = 0.84 for the Chi^2-value.

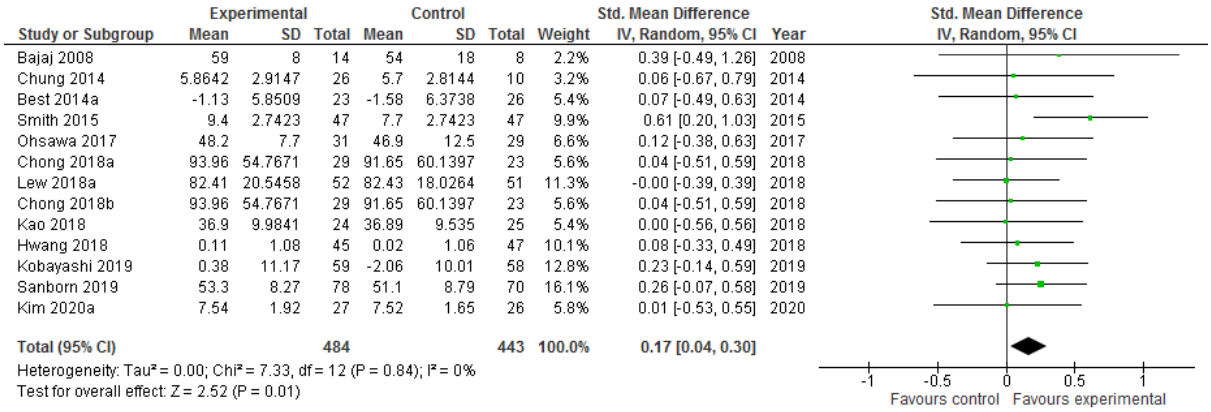

Figure 9: Short term/Working memory forest plot extracted from RevMan.

3.1.5. Delayed memory

Figure 10 shows a pooled effect estimate of 0.17 (95%CI [0.00; 0.33], z = 2.01, p = 0.04) for delayed memory in 296 experimental and 291 control cases. Heterogeneity of the pooled study data shows I^2=0% and p = 0.90.

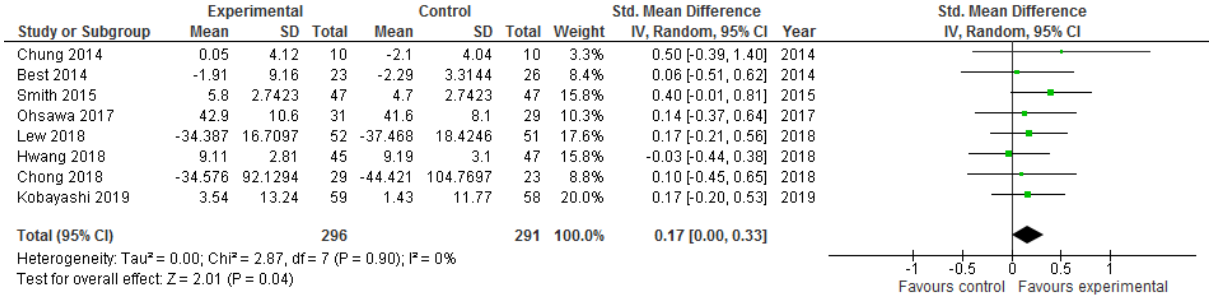

Figure 10: Delayed memory forest plot extracted from RevMan.

3.1.6. Attention/Concentration

Figure 11 shows the total effect estimate of attention/concentration for 533 experimental and 502 control participants as 0.03 (95%CI [-0.11; 0.17], z=0.41, p=0.68). Study data heterogeneity results show I^2=20% and p = 0.23 for the Chi^2-value.

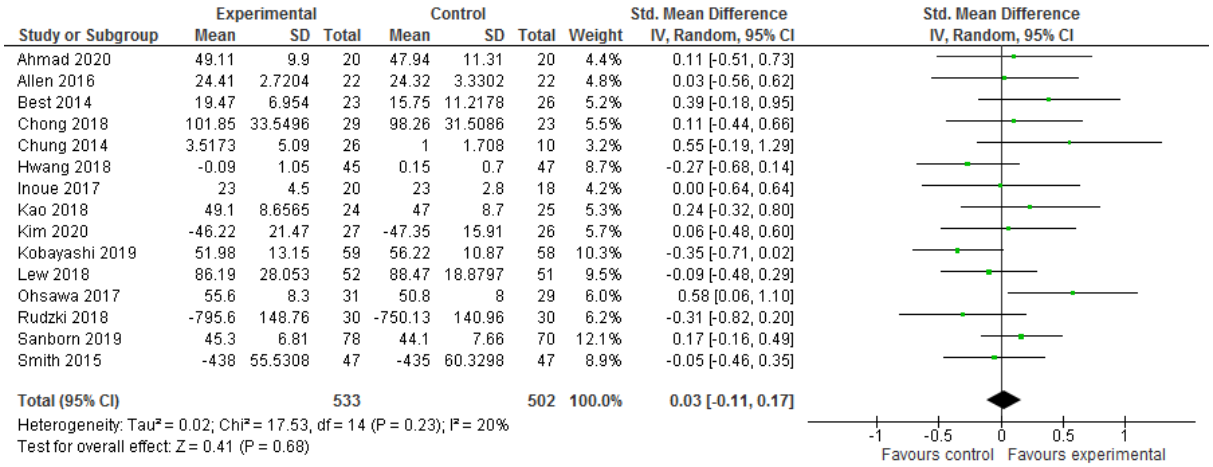

Figure 11: Attention/Concentration forest plot extracted from RevMan.

3.1.7. Verbal learning and language

The pooled effect estimate for verbal learning and language skills can be seen in Figure 12. The overall effect estimate is 0.02 with a 95%CI of [-0.16; 0.2] (z=0.18, p=0.86) and heterogeneity of results data is I^2=26% and p = 0.2 for the Chi^2-value.

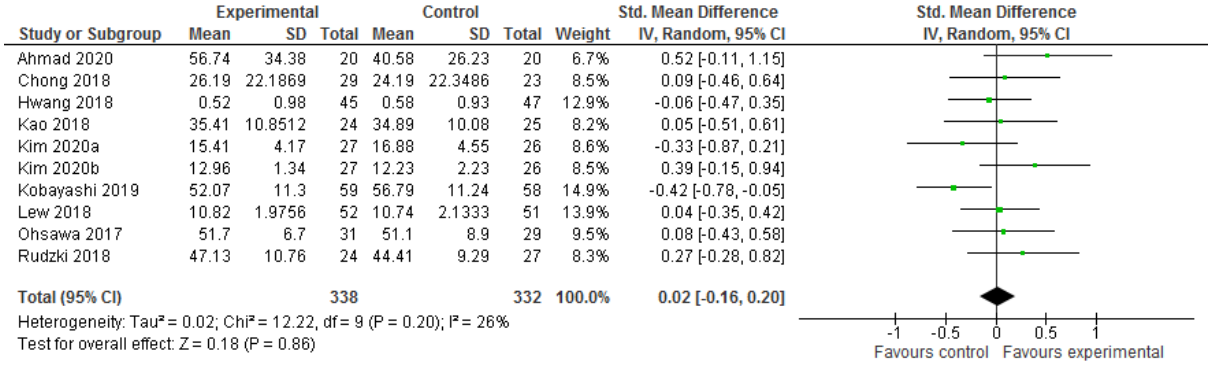

Figure 12: Verbal learning and language skills forest plot extracted from RevMan.

3.2. Summary of forest plot results

Table 3 summarizes the data for the pooled effect sizes of the various separate neurocognitive tests as well as the total cognition pooled effect size.

Table 3: Summary of results

Neurocognitive function	Overall effect estimate (95%CI)	P-value	Heterogeneity (I^2 – value)	Heterogeneity (P-value)
Total cognition	0.15 (0.01; 0.29)	0.03	40%	0.02
(MCI/AD diagnosis)	0.3 (-0.08; 0.67)	0.12	70%	0.002
(No MCI/AD diagnosis)	0.12 (-0.01; 0.24)	0.08	5%	0.39
PAL	0.11 (0.39; 0.7)	0.44	0%	0.42
Processing speed	0.09 (-0.09; 0.27)	0.34	0%	0.62
Short-term/working memory	0.17 (0.04; 0.30)	0.01	0%	0.84
Delayed memory	0.17 (0.00; 0.33)	0.04	0%	0.90
Attention	0.03 (-0.11; 0.17)	0.68	20%	0.23
Verbal learning	0.02 (-0.16; 0.2)	0.086	26%	0.20

For the overall effect size $p<0.05$ was considered to be significant and for heterogeneity $P<0.1$ (for the chi-squire test) or $I^2>40\%$ was considered as significantly heterogeneous to not be coincidental.

4. DISCUSSION

Seeing as the overall effect estimate is according to units of standard deviation rather than units on an outcome scale, according to the rule of thumb, an effect size <0.2 represents a small effect (Higgins & Green, 2011). As can be seen on the results summary (Table 3), all of the pooled effect size estimates for Total Cognition (Figure 4) and the subsets of cognitive tests (Figures 7-12) show a small effect in favour of the experimental (probiotic/ prebiotic/ symbiotic) groups in comparison to the control groups.

Of these cognitive tests, the Total Cognition (Figure 4), Short-term/ Working memory (Figure 9) and Delayed memory (Figure 10) scores were the only scores to yield significant p-values to indicate confidence in the effect size estimates, but there was too much heterogeneity among Total Cognition scores to possibly be coincidental. When taking the heterogeneity into account (Table 3), all individual neurocognitive functions show a heterogeneity score too low to indicate inconsistency of intervention effects across studies. When the Total Cognition sub-analysis according to diagnosis of MCI or AD (Figure 4) sub-totals are taken into account, neither group shows a significant p-value of the pooled estimated effect sizes and it is clear that the heterogeneity among the studies where a MCI or AD diagnosis was made is significant whereas the study results among the studies where an AD or MCI diagnosis was not made was sufficiently homogenous. This was attributed to dissimilarity of methodologies of the individual studies – seeing as further investigation (Figure 5) showed that the heterogeneity among studies using probiotics as supplement was insignificant (I^2 = 0%, p = 0.53) and among the studies using fermented foods as supplements (I^2 = 69% and p = 0.002) the studies were among different ethnic groups, used different fermented supplements, three different methods of measurement were used and one study (Hwang *et al*., 2018) had different baseline composite scores among placebo and experimental groups that changed the direction of the scale. This observation was made again when sub-analysis of Total Cognition scores according to the type of supplement used (Figure 6) was done to determine if pooled result estimates were consistent across all types of supplements. Prebiotic and probiotic studies showed similar effects, although the p-value among the probiotic studies was smaller (0.13 [-0.13; 0.38], p = 0.33 and 0.14 [-0.01; 0.3], p = 0.07 respectively). The fermented food studies had a larger

effect size estimate in favor of the experimental group, but the heterogeneity indicates inconsistency among the included studies.

With regards to the other individual cognitive tests: both the PAL forest plot (Figure 7) and the processing speed forest plot (Figure 8) showed small effect estimates in favor of the experimental group with insignificant heterogeneity among the studies, but, as mentioned, the p-values did not indicate confidence in the effect size estimates. The effect estimates for both attention/concentration and verbal learning and language skills was trivial, as the 95% CI ranges were from -0.11 to 0.17 (Figure 11) and -0.16; 0.2 (Figure 12) respectively, indicating that the effect estimates do not truly favour either group.

5. CONCLUSIONS

It can be concluded that ingestion of probiotics/ prebiotics/ synbiotics may improve short term and delayed memory function, if not total cognition, of patients – regardless of a dementia or AD diagnosis. For greater accuracy of the estimated effect sizes across studies, the baseline risk needs to be taken into consideration. No conclusions can be made regarding long term memory – as it was not included in the outcome measures. Cognitive effects of microbial supplements may take longer periods of time to achieve (most of the included studies for analysis did not exceed 12 weeks of study and only two studies reached 24 weeks of study). For conclusions regarding the longer term use of microbial supplements, cross-sectional studies on populations of high consumers for microbial supplements might be considered. The analysis was limited by the relatively small sample sizes of the individual studies in the analysis. Studies performed on the association between gut health and AD thus far are few and done on a small scale, meaning that the various results could not be applied to a larger population. For a more accurate analysis larger studies may be needed for inclusion, along with unpublished studies.

The results of this study can be substantiated by the results from a meta-analysis by Lv *et al*. (2020), who found that probiotic supplementation had a significant effect in stimulating cognitive function in humans and animals, though the most notable effect was in cognitively impaired people. Healthy individuals showed no significant effect. Another meta-analysis by Deng *et al.* (2020) found that probiotics consumption improved cognition in MCI/AD subjects, but concluded that strong conclusions would be premature, as current evidence is limited and insufficient. Results should be cautiously interpreted and more large-scale, long-period, randomized controlled trials are necessary.

6. Ethical issues – This is a meta-analysis and ethical clearance is not required. Ethical waiver ref: R14/49, protocol no: W-CBP-200817-01.

7. Resource requirements

Internet access

Wits University Library access

All studies and programs used for this analysis are free and available for public download online. This study is feasible within the resources of the applicant.

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APPENDIX A: ETHICS WAIVER

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Ms N Nel
School: Therapeutic Sciences
Department: Pharmancy and Pharmacology
Medical School
University
E-mail: neeskenel@gmail.com

CC: Supervisor: Professor MP Danckwerts <Michael.Danckwerts@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 17/08/2020

REF: *R14/49*

PROTOCOL NO: W-CBP-200817-01 (This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)

PROJECT TITLE: *A meta-analysis of the correlation between dysbiosis and mental health: the effects of probiotics/prebiotics/synbiotics on memory, with a specfic focus on Altzheimer's Disease*

Please find attached the Ethics Waiver Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/ClearScanWaiver.wps

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

17/08/2020

Ref: W-CBP-200817-01

TO WHOM IT MAY CONCERN:

Waiver: This certifies that the following research does not require clearance from the Human Research Ethics Committee (Medical).

Investigator: Ms N Nel
Student No. (if appropriate): 1875925
Staff No. (if appropriate):

Supervisor: Professor MP Danckwerts

School: Therapeutic Sciences
Department: Pharmancy and Pharmacology
Medical School
University

Project title: *A meta-analysis of the correlation between dysbiosis and mental health: the effects of probiotics/prebiotics/synbiotics on memory, with a specfic focus on Altzheimer's Disease*

Reason: Review of information in the public domain.
No human participants will be involved in the study.

CB Penny

Dr CB Penny
Co-Chairperson: Human Research Ethics Committee (Medical)

Research Office Secretariat:
Physical address: Phillip Tobias Building, 3rd Floor, Office 302, Corner York Road and Princess of Wales Terrace, Parktown, Johannesburg 2193.
Postal address: Private Bag 3, Wits 2050
Tel Nos. +27 (0)11-717-1234/2656/2700/1252
Office E-mail: HREC-Medical.ResearchOffice@wits.ac.za.
Website:http://www.wits.ac.za/research/about-our-research/ethics-and-research-integrity/

APPENDIX B: PROOF OF REGISTRATION 2021

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

PROOF OF REGISTRATION

This document certifies the following information about the student's current status at the University of the Witwatersrand, as at 26-Mar-2021.

The student below is registered from January to December for the academic year 2021.

Student Details:

Student ID	: 1875925	Title	: Ms
Surname	: Nel		
Name(s)	: Neeske		
Date of Birth	: 16-Nov-1992	ID Number/ Passport Number	: 9211160131086

DATE FIRST REGISTERED: 10-Jan-2018

Programme of Study Details:

Program Code and Title	: MCA08 - Master of Science in Medicine
Programme Type	: Masters (C) Degree
Attendance Type	: Part-Time
Date of Registration	: 15-Mar-2021
Year of Study	: Year of Study 2

Courses Registered for in 2021:

COURSE	COURSE DESCRIPTION	SESSION	STATUS
PACY7026A	Research Report	Full Year	Enrolled

FACULTY REGISTRAR