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Prebiotics and synbiotics effect on CNS-related conditions in humans: a systematic review

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Master's degree (MSc) in Food Science – Nutrition, Biomedicine and Health

Acknowledgements

This master thesis marks the end of my education in Food Science – Nutrition, Biomedicine and Health by the Faculty of Chemistry, Biotechnology and Food Science at the Norwegian University of Life Sciences (NMBU, campus ÅS). With my bachelor's degree in Nutrition and soon master´s degree in Food Science, I have gained an increased interest in the connection between nutrition and health, and especially the role of the gut microbiota.

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Sammendrag

Den viktige rollen av tarmens mikrobiota i menneskers helse er allerede godt etablert og forskere har fått en økt interesse for dette området over de siste årene. Flere studier har foreslått at en endret sammensetning av tarmens mikrobiota er nøkkelen til endringer i tarm-hjerne-aksen. En måte å endre sammensetningen av tarmens mikrobiota på, er ved inntak av prebiotika og synbiotika. Rollen av prebiotika og synbiotika i kommunikasjonen mellom tarmen og hjernen, og effekten på mental helse og kognitiv funksjon, har vist lovende resultater i flere dyrestudier. Men, det er begrenset evidens for prebiotika og synbiotikas effekt på hjernen hos mennesker.

I denne oppgaven ble det utført et systematisk søk for å undersøke evidensen av prebiotika og synbiotikas effekt på neurologiske og psykologiske tilstander. Søket ble utført i databasen PubMed i januar 2023 med ulike kombinasjoner av søkeordene: prebiotika, kognitiv funksjon, og RCT.

Tilskudd av prebiotika og synbiotika demonstrerte en lovende effekt på mental helse, spesielt angst og depresjon, samt kognitiv funksjon i mennesker. Denne effekten ser ut til å bli påvirket av en rekke faktorer slik som individuelle forskjeller i tarmens mikrobiota, patologisk status og type, dose og lengde av intervensjon, for å nevne noen få. Tilskudd av prebiotika og synbiotika kan også føre til en forbedring i metabolske og inflammatoriske biomarkører. Disse funnene foreslår at tilskudd av prebiotika og synbiotika kan påvirke deres effekt på psykiske helseparametere. Sammensetningen av tarmens mikrobiota spiller også en viktig rolle i toveis kommunikasjonen mellom hjernen og tarmen. Det var ikke mulig å forklare mekanismene bak prebiotika og synbiotikas effekt på tarm-hjerne-aksen, da disse mekanismene ikke er godt nok etablert. Typen, dosen og lengde på intervensjon nødvendig for den fordelaktige effekten av tilskudd av prebiotika og synbiotika er fremdeles usikker. Videre forskning er derfor nødvendig for å undersøke den mulige bruken av prebiotika og synbiotika som terapeutiske midler.

Abstract

The important role of the gut microbiota in human health is already well established, and researchers have grown an increased interest in this subject over the past years. Various studies have suggested that an altered composition of the gut microbiota is key to the modulation of the gut-brain-axis. One way to alter the composition of the gut microbiota is by intake of prebiotics and synbiotics. The role of prebiotics and synbiotics in communication between the gut and the brain, and the effect on mental health and cognition, has shown promising results in several animal studies. However, there is limited evidence for the prebiotic influence on the human brain.

In this thesis, a systematic search was conducted to investigate the evidence for prebiotics effect on neurological and psychiatric disorders. The search was conducted in the database PubMed in January 2023 with different combinations of the search terms: prebiotics, cognitive function, and RCT.

Prebiotic and synbiotic supplementation demonstrate a promising beneficial effect on mental health, especially anxiety and depression, and cognitive function in humans. This effect, however, seems to be influenced by various factors such as individual differences in gut microbiota, pathological state, and type, dose and length of intervention, to mention a few. Prebiotic and synbiotic supplementation may also lead to an improvement in metabolic and inflammatory biomarkers. These findings propose that prebiotic or synbiotic supplementation may influence their effects on mental health parameters. The composition of the gut microbiota also plays an important role in the bidirectional communication between the brain and the gut. It was not possible to explain the mechanisms behind prebiotics and synbiotics effect on the gut-brain-axis, as these mechanisms are not well established. Moreover, the type, dose, and duration of intervention necessary to provide a beneficial effect of prebiotic and synbiotic supplementation is still uncertain. Future research is therefore needed to investigate the potential use of prebiotics or synbiotics as therapeutical agents.

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Abbreviations

RCT: randomized controlled trial
FOS: fructooligosaccharides
GOS: galactooligosaccharides
RS: resistant dextrin
LS: lactosucrose
PDX: polydextrose
CSO: camelina sativa oil
SCFA: short chain fatty acid
CNS: central nervous system
HPA-axis: hypothalamic-pituitary adrenal axis
GF: germ free
MS: mass spectrometry
BMI: body mass index
WC: waist circumference
BDNF: brain derived neurotrophic factor
SCFA: short-chain fatty acids
TNF: tumor necrosis factor
DAO: diamine-oxidase
BDNF: brain derived neurotrophic factor
Hs-CRP: high-sensitivity C-reactive protein
VCAM-1: vascular cell adhesion molecule 1
IL: interleukin
IFN: interferon
GABA: γ-aminobutyric acid
ACTH: adrenocorticotropic factor
KYN: kynurenine
TRP: tryptophan
LPS: lipopolysaccharides
IFABP: intestinal fatty-acid binding protein
CRH: corticotropin-releasing hormone
SBP: systolic blood pressure
DBP: diastolic blood pressure
LDL: low density lipoprotein
HDL: high density lipoprotein
TG: triglycerides
TC: total cholesterol
BCAA: branched chain amino acid
NMDAR: N-methyl-D-aspartate receptor
FPG: fasting plasma glucose
FBS: fasting blood sugar
RMR: resting metabolic rate
HOMA-IR: homeostatic model assessment for insulin resistance
HbA1c: hemoglobin A1c
MDA: malondialdehyde
TAC: total antioxidant capacity
SOD: superoxide dismutase

DDP-IV: dipeptidyl-peptidase IV
ADHD: attention deficit hyperactivity disorder
T2M: type 2 diabetes
CAD: cardiovascular disease
GI: gastrointestinal
ESRD: end-stage renal disease
HD: hemodialysis
HE: hepatic encephalopathy
NAFLD: non-alcoholic fatty liver disease
IBS: irritable bowel syndrome
IBD: irritable bowel disease
DASS-21: Depression, Anxiety, and Stress scale 21
TMT: Trail Marking Test
ICT: Inhibitory Control Test
RVP: Rapid Visual Information Processing
IED: Intra-Extra Dimensional Set Shift
SF-36: Short Form 36
GDS-15: Geriatric Depression Scale 15
MMSE: Mini-Mental Status Examination
DERS-16: Difficulties in Emotion Regulation Scale 16
BDI: Beck Depression Inventory
STAI: State-Trait Anxiety Inventory
HADS: Hospital Anxiety and Depression Scale
SCQ: Social Communication Questionnaire
AQ: Autism Spectrum Quotient
ITT: Intention-To-Treat
PP: Per-Protocol
SCL-90-R: Symptom CheckList-90-Revised
GHQ: General Health Questionnaire
BACS: Brief Assessment of Cognition in Schizophrenia
GSES: Global Self-Efficacy Scale
MADRS: Montgomery Asberg Depression Rating Scale
HDRS: Hamilton Depression Rating Scale
PSQI: Pittsburgh Sleep Quality Index
PSS: Perceived Stress Scale
SMFQ: Children Mood and Feeling Questionnaire
CSHQ: Child Sleep Habits Questionnaire
PANAS: Positive and Negative Affect Schedule
SPANE: The Scale of Positive and Negative Experience
PEC: Profile of Emotional Competence

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1 Introduction

Over the past years there have been compelling evidence for a link between the gut microbiota and neuropsychological disorders. Various studies have suggested that an altered composition of the gut microbiota is key to the modulation of the gut-brain-axis. One way to alter the composition of the gut microbiota is by intake of prebiotics or synbiotics (Cryan et al. 2019, Sergeev et al. 2020). Prebiotics are complex carbohydrates, naturally found in the diet or commercially available as dietary supplements with the purpose of providing a health benefit for the host. Prebiotics synergistically combined with probiotics are termed synbiotics. Synbiotics act synergistically due to the prebiotic selectively favoring a probiotic microorganism (Cencic et al. 2010). After oral intake, prebiotics end up, more or less undigested, in the large intestine where they are metabolized by bacteria (Cryan et al. 2019). Besides the resulting altered bacterial composition, it also leads to production of favorable metabolites that affect the host in a beneficial way. These metabolites are believed to have an important role in the communication between the gut and the brain. Even though several lines of evidence suggest an impact of microbiota on the brain, the mechanisms that underlie these effects are still unknown.

The communication between the gut and the brain has been demonstrated in several animal studies (Burokas et al. 2016, Hoffman et al. 2019, Zhou et al. 2012, Lyte et al. 2016). However, there is limited evidence for the prebiotic and synbiotic influence on the human brain. The aim of this thesis is therefore to review randomized controlled trials done in humans who have studied the effect of prebiotics and synbuiotics on clinical parameters in the brain to systematically consider the status on the field today.

1.1 Dietary fibers and prebiotics

The human diet consists of a variety of plants in the form of fruits, vegetables, nuts and cereals. A major part of many plant foods are carbohydrates that are digested and absorbed in the small intestine. A significant fraction of the plant is, dietary fiber, which are not digested but end up in the colon, where a number of dietary fibers undergo microbial fermentation (El Kaoutari et al. 2013). The major components of dietary fiber are cell wall polysaccharides (Lovegrove et al. 2017). The gut microbiota consists of enzymes able to hydrolyze the chemical bonds within some dietary plant fibers, examples of such fibers are inulin-type fructans, galactooligosaccharides (GOS), resistant starch (RS), and certain soluble hemicelluloses to mention a few (El Kaoutari et al. 2013, Gill et al. 2020). All these fibers have a high

fermentability, meaning that colonic bacteria can utilize these fibers to sustain growth and energy needs. In the colon, this metabolism is largely anaerobic and important byproducts from the fermentation are short chain fatty acids (SCFAs). Overall, dietary fibers impact the gastrointestinal tract in several ways, many of them beneficially due to their effects on digestion and absorption, improved glycemic and lipemic responses, reduction in plasma cholesterol through limiting bile salt reabsorption, by influencing gut transit, and microbiota growth and metabolism (Gill et al. 2020). Furthermore, dietary fiber has been linked to reduced incidence of gut disorders and diseases like irritable bowel syndrome (IBS), inflammatory bowel disease (IBD) and diverticular disease.

The recommended dietary intake of fiber is 25 and 35 g per day for females and males, respectively. However, the average intake of dietary fiber by adults across the world is typically below 20 g per day (Mayor, 2019). Examples of good dietary sources of fibers are fruits, vegetables, legumes, and foremost whole grain cereals of oat, wheat, and barley (Gill et al. 2020). Dietary fibers fermented by bacteria in the gastrointestinal tract will impact the composition of the microbiota and microbial metabolic activities (Holscher, 2017). The impact fiber consumption will have on the gastrointestinal microbiota depends on the type of fiber consumed and its physiochemical properties (solubility, viscosity and fermentability), as well as the fiber dosage, and the microbial composition in the individual consuming the fiber. The location of fiber fermentation in the gastrointestinal tract will depend on the degree of polymerization as well as the solubility of carbohydrate polymers. Solubility refers to the dietary fibers ability to be dissolved in water.

The final definition of dietary fiber adopted in 2009 from the Codex Committee for Nutrition and Foods for Special Dietary Uses (CCNFSDU) states: “dietary fiber means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belonging to the following categories: (1) edible carbohydrate polymers naturally occurring in the food as consumed, (2) carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, and (3) synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

Many dietary fibers are classified as prebiotics (Stephen et al. 2017). Examples of such dietary fibers are inulin, fructooligosaccharides (FOS), GOS and RS. Prebiotics are mainly

carbohydrates, but the term is not restricted to only carbohydrates (Bindels et al. 2015). It also includes non-carbohydrate compounds that are metabolized by microorganisms in the gut and have a potential role of modulating the gut microbiota in a way that provides health benefits for the host. An example of such prebiotics are polyphenols, which provide health benefits mainly due to their antioxidant and anti-inflammatory properties (Plamanda et al. 2022). Polyphenols can be found naturally in cereals, fruits, vegetables, wine, coffee, and tee. It has been demonstrated that administration of polyphenols in mice can increase the levels of *Bacteroidetes, Firmicutes, Proteobacteria,* and *Actinobacteria,* and decrease the levels of tumor necrosis factor (TNA)-α, interleukin (IL)-1, and IL6 (Li et al. 2019). However, this review will focus on assessing dietary fibers classified as prebiotics. Prebiotics are naturally found in our diet but can also come in the form of dietary supplements. Prebiotics have their role in the colon, where it is utilized by members of the gut microbiota and will only be considered functional when it stimulates the growth and activity of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* (Zaman et al. 2015). The health benefit received depends on the type of prebiotic dietary fiber administered (Carlson et al. 2018). Some prebiotic dietary fibers have evidence that they promote a beneficial effect for the host. These are β-glucan, FOS, oligofructose, and inulin, GOS, isomaltooligosaccharides, guar gum, lactulose, RS, and resistant maltodextrin, and xylooligosaccharides and arabinooligosaccharides. However, there are most evidence for the health benefits of FOS, inulin, and GOS.

There have been different definitions of the prebiotic concept over the years. The first definition described prebiotics as “nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” (Gibson et al. 1995). The 2010 definition by Gibson et al. was as follows: “a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (Gibson et al. 2010). A new definition was proposed by Bindels et al. in 2015: “a nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host” (Bindels et al. 2015). Even though dietary fibers and prebiotics share several characteristics, classification as a prebiotic requires specificity.

There is high potential attributed to the combined treatment of probiotics and prebiotics which leads to the term “synbiotic”, used to describe the synergistically acting probiotics and prebiotics (Cencic et al. 2010). They act synergistically due to the prebiotic selectively favoring

a probiotic microorganism. A commonly used synbiotic is the combination of the probiotic strains *Bifidobacterium* or *Lactobacillus* genus with the prebiotic FOS (Markowiak et al. 2017). It has been demonstrated that synbiotic supplementation modulates the gut microbiota as observed by an increased abundance of *Bifidobacterium* and *Lactobacillus* (Sergeev et al. 2020). It is therefore evidence that both prebiotics and synbiotics have the ability to modulate the gut microbiota.

1.2 The gut microbiota

The gut microbiota is a term used to describe the variety of commensal microorganisms that inhabit the gastrointestinal tract and are essential to human health (Shreiner et al. 2015). The gut microbiota consists of bacteria, archaea, viruses, and eukaryotic microbes. These commensal microbes are important for metabolic functions, education, and development of host immunity, and protect against pathogenic microbes. The bacterial phyla with the highest abundance in healthy adults are Firmicutes (64%) including genera *Lactobacillus, Bacillus, Clostridium, Enterococcus, Ruminococcus, Eubacterium, Faecalibacterium* and *Roseburia,* as well as Bacteroidetes (23%) including genera *Bacteroides* and *Prevotella* (El Kaoutari et al. 2013, Ramos et al. 2021). High numbers of Actinobacteria and Proteobacteria members are also present. However, the proportions of these phyla vary great between individuals and depends on factors like geographical, lifestyle and temporal variations, and disease. There is also a number of factors that can influence the composition of the gut microbiota early in life. These are factors like infection, mode of delivery (caseation or vaginal), use of antibiotics, the nature of nutritional provision, environmental stressors, and host genetics (Cryan et al. 2019). Diet also has a remarkable impact on the gut microbiota (Ramos et al. 2021). The absorption and metabolism of nutrients can be influenced by the composition of the gut microbiota, which in turn can affect host physiology. Moreover, nutrients, different foods, bioactive compounds, and dietary patters can also change the composition of the gut microbiota with either a beneficial or detrimental effect on human health.

With the great impact the gut microbiota has on human health, there has been a growing interest in studying disease related to changes in the microbiota (Shreiner et al. 2015). It is speculated that dysbiosis, which often refers to a dysregulated and “unbalanced” microbiota composition, may lead to disease but the disease itself may also cause adverse changes in the microbiota (Messer et al. 2018). Dysbiotic imbalance in the community of the gut microbiota may be caused by the gain or loss of members of the microbiota or changes in relative abundance of

microbes. Differences in the gut microbial communities have been identified in patients with gut disorders compared with healthy controls. However, it has been observed a wide variety in the specific microbial taxonomic differences that makes it challenging to identify a definite disease associated community structure. It is therefore unclear how to define dysbiosis and the relationship between dysbiosis and disease is not fully understood. The Anna Karenina Principle based on Leo Tolstoy´s assertion: "All happy families are all alike; each unhappy family is unhappy in its own way" can be applied to the gut microbiota and states that a healthy microbiota is somewhat stable and alike, whereas an "unhappy" microbiota can come in many variants.

To understand how the gut microbiota can affect the health of the host, it is important to note that the gut microbiota is critical for digestion to provide nutrients from substrates that the host otherwise would not be able to digest (Shreiner et al. 2015). The communication between the microbiota and the immune system is essential for the hosts health. Through this interaction the immune system learns to tolerate the commensal microbiota and respond to pathogens when necessary. The gut microbiota is in turn responsible for educating the immune system. In the last few years, it has been revealed that the gut microbiota not only affects the gastrointestinal tract (GI tract) (Silva et al. 2020). The gut microbiota also plays a role in the bidirectional communication between the GI tract and the central nervous system.

1.2 Prebiotics and effects on microbiota and health

1.3.1 Galactooligosaccharides and fructooligosaccharides

FOS and GOS are both oligosaccharides with the ability to escape digestion and further be fermented in the colon to produce SCFAs. FOS is mainly found in plants whereas GOS is mainly found in milk (Cryan et al. 2019). FOS consists of linear chains of fructose units ranging from two to 60, linked by β-(2-1) bonds (Sabater-Molina, 2009). GOS contains galactose units and their chemical structure vary by chain length, branching, and glycosyl linkages (Mei et al. 2022). GOS can be divided into α-GOS and β-GOS based on the different galactosidic bonds attached.

A study carried out by Burokas et al. found that FOS and GOS, alone and in combination, had a positive effect on behavior and brain chemistry related to anxiety and depression in mice (Burokas et al. 2016). The prebiotics led to differences in microbiota diversity and higher levels of SCFAs in the cecum. Intake of FOS+GOS prevented the deleterious effects on behavior,

cytokine release, and microbiota induced by chronic psychosocial stress. Moreover, prebiotic intake reduced stress-induced plasma corticosterone levels, the effect was higher with FOS+GOS. There was also an observed reduction in anxiety as measured with the open field and elevated maze tests. The highest effect was observed for FOS+GOS. The same results were shown for depression-like behavior as measured with tail suspension and forced swim tests.

For the microbial diversity, the study especially found an increase in the abundance of *Akkermansia* (Burokas et al. 2016). The abundance of *Bacteroides* was also increased after prebiotic intake, which was related to an increase of propionate levels. The mice who received FOS+GOS showed higher levels of BDNF expression in the hippocampus. This group also showed an increase in mRNA for a subunit of the γ-aminobutyric acid $(GABA)_B$ receptor in the hippocampus. FOS+GOS intake was also tested in mice subjected to chronic stress. Intake of FOS+GOS attenuated acute stress-induced corticosterone levels and hyperthermia in chronically stressed mice. FOS+GOS normalized the increased proinflammatory response caused by chronic social stress. Furthermore, FOS+GOS protected the microbiota from the chronic stress. Stress led to a decrease in the *Actinobacteria:Proteobacteria* ratio, but the prebiotics normalized the ratio. FOS+GOS also prevented the reduction of *Bifidobacterium* and *Lactobacillus* caused by chronic stress. In humans, administrations of FOS and GOS have been shown to increase the relative abundance of *Bifidobacterium* and reduce the butyrate-producing bacteria (Liu et al. 2017).

1.3.2 Inulin

Inulin is mainly found in fruits, vegetables, and wheat (Cryan et al. 2019). It is a water-soluble storage polysaccharide commonly used as a prebiotic. Inulin consists of fructose units linked by β-(2-1)-D-frutosyl fructose bonds (Shoaib, 2016). The functional properties of inulin are influenced by the degree of polymerization (DP) and branches. Inulin in plants have a relatively low DP, whereas inulin in bacteria have a high DP.

It has been demonstrated that a combination of inulin and oligofructose (16 g/d) administered for 6 weeks can lead to an increase in bifidobacteria, total SCFA, acetic acid and propionic acid in patients with type 2 diabetes (Birkeland et al. 2020). However, an effect on the microbial diversity was not observed. Hoffman et al. found that administration of inulin in mice led to an increased abundance of *Prevotella* and *Lactobacillus sp* as well as a decrease in the harmful bacteria *Escherichia, Turicibacter* and *Proteus* (Hoffman et al. 2019). These changes in the microbiota may relate to improved immune function. Moreover, there was an observed increase

in the levels of SCFAs, tryptophan-derived metabolites, bile acids, glycolytic metabolites, and scylloinositol. Inulin also showed an effect on the hippocampus by reducing brain inflammation characteristics of early Alzheimer's disease. A clinical trial conducted in elderly humans did not find an effect on cognitive functions after supplementation with the prebiotic's inulin and FOS (Buigues et al. 2016).

1.3.3 Resistant starch

Starch (amylose and amylopectin) is an important storage carbohydrate in plants and an important source of energy in the diet (Lovegrove et al. 2017). Available starch is digested in the small intestine, RS on the other hand is not digested but fermented in the colon by members of the gut microbiota. The fermentation of RS leads to a health impact on the host, mostly due to enhanced butyrate production (DeMartino et al. 2020). Fermentation of RS will also lead to the production of the other SCFAs, namely acetate and propionate, but butyrate is increased the most. However, there are individual differences in the hosts microbiota as well as various types of RS that will determine the health benefits one will receive from ingestion of RS. RS can be classified into five types: (1) the physically inaccessible RS1, (2) native starch granules termed RS2, (3) retrograded starch RS3, (4) chemically modified starch RS4, and (5) starch able to form complexes between amylose and long branch chains of amylopectin with lipids RS5 (Lovegrove et al. 2017).

Because of their ability to escape digestion in the small intestine and be fermented in the colon, RS has the potential to be used as a prebiotic (Zaman et al. 2015). To be used as a prebiotic, RS must fulfill three criteria: (1) resistance to the upper gastrointestinal environment, (2) fermentation by the intestinal microbiota, (3) and selective stimulation of the growth and/or activity of the beneficial bacteria. Previous studies have shown that the administration of RS as a prebiotic can have a beneficial effect on behavior in rodents (Zhou et al. 2012, Lyte et al. 2016).

1.4 Gut-brain axis and possible roles of dietary fibers and prebiotics

Over the last few years, there have been compelling evidence for a link between the gut microbiota and brain function (Chen et al. 2021). The gut-brain axis includes the central nervous-, endocrine-, and immune system and is a bidirectional communication system between the microbiota and the brain. The gut-brain axis may be modulated due to an altered

composition of enteric microbial communities (Kao et al. 2016). The gut microbiota contains a range of species that produce different neurotransmitters by catabolizing ingestible compounds. Examples of such neurotransmitters are glutamate, GABA, serotonin, and dopamine (Chen et al. 2021). Neurotransmitters produced in the gut act on the brain via the blood circulation, the enteric nervous system, and the vagus nerve. Their role is to carry messages between neurons via synapses to control behaviors like emotion, memory, etc. The communication mechanisms also include the tryptophan metabolism and the hypothalamic-pituitary-adrenal (HPA) axis, and microbial metabolites such as SCFAs, branched chain amino acids, and peptidoglycans (Cryan et al. 2019, Silva et al. 2020). Dysbiosis of the gut microbiota has been associated with both neurological and psychiatric disorders such as autism spectrum disorder, depression, and anxiety (Shreiner et al. 2015). Intake of prebiotics have a beneficial role in increasing the abundance of beneficial gut bacteria. However, Taylor et al. found that there was insufficient evidence for the effect of prebiotics on mood disorders due to a lack of studies (Taylor et al. 2018). They stated that there was not enough clinical data to causatively link the gastrointestinal microbiota to depression, anxiety, and stress in humans. Synbiotic supplementation is also suggested to have a beneficial role in mental health and cognition. A study conducted in piglets found that synbiotic supplementation may have a beneficial effect in cognitive function (Parois et al. 2021).

1.4.1 Potential mechanisms describing the gut-brain-axis

Even though several lines of evidence suggest an impact of microbiota on the brain, the mechanisms that underlie these effects are still unknown. Metabolites produced by microbiota are believed to be the key for communication between the gut and the brain. A comparison of plasma metabolites between germ-free (GF) and conventional (conv) mice using mass spectrometry (MS) revealed for instance that tryptophan levels were highest in GF mice, and serotonin was highest in conv mice (Wikoff et al. 2009). These data support that the microbiota has a role in the metabolism of dietary tryptophan to serotonin. Moreover, metabolomics assessments in conv mice showed higher fecal levels of norepinephrine, epinephrine, and the dopamine precursors tyramine and L-DOPA compared with GF mice (Lai et al. 2021). The conv mice also had elevated glutamate, GABA, and serotonin. The findings from Wikoff et al. suggest that the microbiota plays an important role in neurotransmitter metabolism in the gut in terms of production, transformation, and bioavailability.

Psychological disorders such as depression and anxiety and different acute or chronic CNS disorders such as Alzheimer's disease, Parkinson's disease, and major depression are associated with neuroinflammation (Calabrese et al. 2014). Neuroinflammation will in turn negatively influence hippocampal gene expression of the brain derived neurotrophic factor (BDNF). Prebiotics have been shown to affect the expression of BDNF, a protein that controls different aspects of survival, development, and function of neurons in both the peripheral and the central nervous system (CNS) (Kao et al. 2016). A lack of gut bacteria reduces the expression of BDNF and N-methyl-D-aspartate receptor (NMDAR) subunits (Savignac et al. 2013). However, administration of prebiotics in rats increased brain BDNF expression. For central NMDAR signaling the effect was greater for GOS than for FOS. A systematic review and meta-analysis conducted in humans revealed that supplementation with synbiotics significantly increased the levels of BDNF and suggested a beneficial effect on the brain (Foshati et al. 2022). It has been hypothesized that BDNF modulation could be involved in the mechanisms by which inflammation may affect brain function (Calabrese et al. 2014).

SCFAs are monocarboxylic acids with a chain length from one to six carbon atoms and are the primary end-products of fermentation of dietary fibers (Silva et al. 2020). It is comprised mainly of acetate, propionate, and butyrate, present in a molar ratio of 60:20:20, respectively (Takagi et al. 2016). SCFAs are produced in the colon before they enter the colonocytes, the liver, or the systemic circulation. These metabolites are further responsible for the changes in the enteric environment to the brain (Holscher, 2017, Kao et al. 2016). Prebiotic intake leads to the production of SCFAs, which may in turn interact with enteric immunomodulatory cells and further influence brain function. The majority of the SCFAs produced in the intestine are absorbed in the periphery. However, evidence suggests that a small amount can cross into the CNS. Baxter et al. found that both RS prepared from potatoes (RPS) and inulin significantly increased total SCFA concentrations in humans (Baxter et al. 2019). Administration of RPS increased butyrate and acetate concentrations. Inulin did not lead to a significant difference in butyrate levels. The total SCFA concentrations increased after inulin administration, but no significant differences were found in individual SCFAs. Increased production of SCFAs may be beneficial for the human health (Blaak et al. 2020). However, current data are mostly based on animal studies.

1.5 Purpose and research question

The important role of the gut microbiota in human health is already well established, and there have been compelling evidence for a link between the gut microbiota and the brain, namely the gut-brain axis (Silva et al. 2020). A majority of the intervention studies today is carried out in animals (Burokas et al. 2016, Hoffman et al. 2019, Zhou et al. 2012, Lyte et al. 2016). These studies have found promising results that prebiotic administration modulates the composition of the gut microbiota in a way that beneficially affects the brain. One study also found that synbiotic supplementation led to an improvement in cognitive status in piglets (Parois et al. 2021). However, there is limited evidence for the prebiotic and synbiotic influence of the human brain.

The purpose of this thesis is therefore to carry out a systematic review to better map the effect of prebiotics and synbiotics on neuropsychological disorders in humans. This is desirable to get a better understanding of the communication mechanisms between the gut and the brain, and further to investigate the possibility of the use of prebiotics as therapeutical agents in brain-related diseases.

In the search for a better understanding, the following research questions are formulated:

> "How does supplementation with prebiotics affect mental health and cognitive measurements in humans?"
> "How does supplementation with synbiotics affect mental health and cognitive measurements in humans?"

2 Method

A systematic search was carried out in PubMed January 1st, 2023. The search was limited to include only randomized controlled trials (RCT's). Table 1 presents the search terms used regarding the subjects: prebiotics, cognitive function, and RCT. The search terms within each group were combined with the Boolean operator "or" and search words between groups were combined with the Boolean operator "and". Table 2 presents inclusion- and exclusion criteria for the search. The first search gave a total of 169 articles. Figure 1 presents the results of the systematic search with the selection of included articles.

Table 1. Search words used in the systematic search.

	Prebiotic	Cognitive function	Randomized controlled trial
	Prebiotics	Behavior	
	Inulin	Brain	
OR		Alzheimer	
		Dementia	
		Anxiety	
	AND		

Table 2. Inclusion- and exclusion criteria used for selection of relevant articles.

Inclusion criteria	Polysaccharides classified as prebiotics
	Randomized controlled trial
	Human intervention
Exclusion criteria	Infants

The search was restricted to include only RCT's, polysaccharides classified as prebiotics, and human interventions. Articles studying infants were excluded. The inclusion- and exclusion criteria were set to ensure that the number of articles included were manageable, and that articles not relevant to this study were excluded.

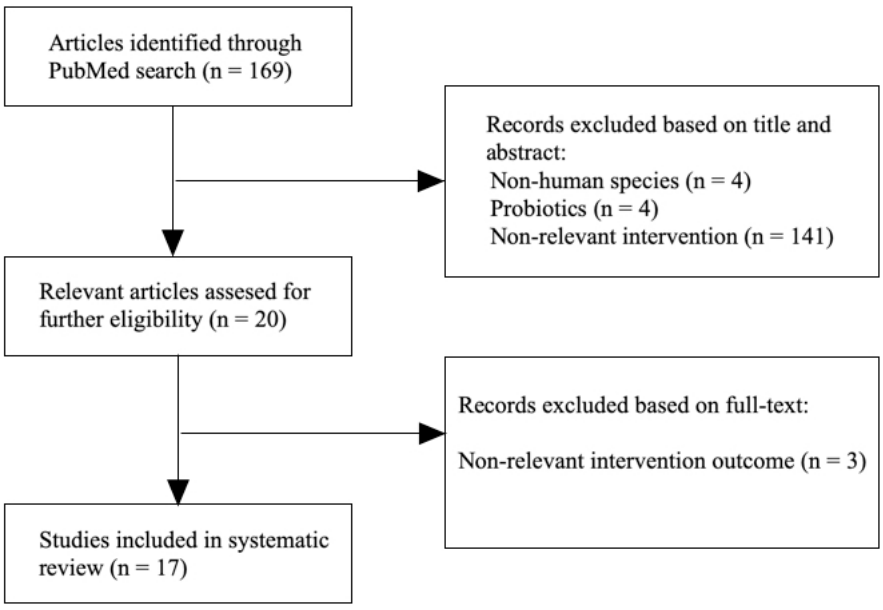

Figure 1. Result of systematic search and selection of included studies.

The PubMed search resulted in a total of 169 articles where titles and abstract were read before exclusion. To ensure an adequate amount of research it was decided to also include articles who reviewed the effect of synbiotics, and not only prebiotics alone. Articles who investigated the effect of probiotics alone were excluded. After exclusion, there was a total of 20 articles

assessed for further eligibility. The full articles were read more than once, and three additional articles were excluded. The exclusion was based on the intervention outcome, where the articles did not have an outcome related to cognitive function.

A systematic search has the advantage of reducing the chance of bias, and it makes it possible to identify gaps in existing research. However, a well-constructed search strategy is crucial, especially when selecting the search terms. There is a variety of different terms relating to prebiotic and cognitive function. These may be different types of prebiotics, or different diseases or conditions related to cognitive function. However, it was decided to use the most common terms within this field to ensure a manageable amount of research. It was decided later in the process to also include studies on synbiotics. The term was therefore not included in the original search and the search was not changed at a later stage. It is therefore likely that more results would have appeared if the search term synbiotics was included in the search.

3 Results

The 17 included articles are RCT's who have reviewed prebiotics (10 studies) or synbiotics (seven studies) on cognitive function and mental health. Of the 10 studies with prebiotic administration the effects were assessed in participants with various underlying disorders such as metabolic disorders (four studies), two studies with depression, one with psychosis, one with illiteracy, one with a combination of metabolic disorder and depression, and the last one with healthy participants. The seven studies on synbiotcs also included participants with different underlying disorders such as obesity, pre-frail elderly, ADHD, hepatic encephalopathy, cardiovascular disease, athletes versus sedentary participants, and lastly end-stage renal disease. In both the prebiotic and synbiotic studies the supplementations were orally administered. Out of the seven studies who assessed the effect of synbiotics, one study examined the combined effect of synbiotcs and BCAA. The distribution of the articles is presented in table 3 and 4. Table 3 summarizes prebiotics effect on mental health and cognition, whereas table 4 summarizes synbiotics effect on mental health and cognition. Division of the studies based on the type of prebiotic or synbiotic received is presented in table 5.

3.1 Prebiotics effect on the brain

3.1.1 Prebiotics effect on mental health

A total number of ten studies have looked at the effect of prebiotics on mental health. The prebiotics used in these studies were inulin, resistant dextrin, GOS, β-GOS, 4G-β-D-Galactosucrose (LS), Nutriose®06 FM, and Litesse®Ultra.

In the first four studies, participants suffered from metabolic disorders. In the first study by Leyrolle et al. they included obese participants aged 18-65 years with a BMI > 30kg/m^2 and the presence of at least one metabolic obesity-related disorder (Leyrolle et al. 2021). The participants were chosen because obesity often can be related to metabolic and behavioral diseases. The study aimed to analyze if administration of 16 g inulin per day for a period of 12 weeks could be linked to gut microbiota changes occurring upon prebiotic intake and the effect on mood and cognition. The intervention group were also advised to consume vegetables rich in inulin together with a calory restricted diet. Out of 106 participants, 94 underwent psychological assessments and 86 had both their gut microbiota sequenced and were scrutinized in a panel of behavioral tests. The behavioral tests were aimed at measuring Profile of Emotional Competence (PEC TOT) and The Scale of Positive (PE) and Negative Experience (NE) (SPANE). Three months inulin supplementation led to a moderate improvement in emotional competence (PEC TOT) and a significant decrease in negative emotion (SPANE NE) when comparing baseline with the end of the study in the prebiotic group ($p<0.001$). Furthermore, they wanted to identify participants whose moods could benefit from inulin by testing if baseline bacteria composition and abundance could predict the effect of inulin supplementation on psychological parameters.

The prebiotic group were therefore divided into those that responded to inulin (positive responders) and those that did not (negative responders) with respect to change in positivity score (difference between positive and negative affect as measured by Positive and Negative Affect Schedule, PANAS). The baseline levels of *Coprococcus* and *Lactobacillus* were significantly different between positive and negative responders. *Lactobacillus* was present only in 41%, whereas *Coprococcus* was present in over 90% of the participants. It was therefore concluded that the level of *Coprococcus* was more suitable to predict the behavioral response toward inulin supplementation. The positive responders also had a larger increase of *Bifidobacterium* and *Haemophilus,* and a difference in biological parameters was observed after intervention. It was observed a significant increase in IL-8 and a greater decrease in dipeptidyl-peptidase IV (DDP-IV) (known to degrade GLP-1), and subcutaneous fat mass in positive responders compared with negative responders. There were no significant differences for other

immunological markers. The study revealed a significant correlation between the positivity score and *Bifidobacterium, Haemophilus,* IL-8, and subcutaneous fat mass. It was concluded that intake of inulin in obese subjects led to a moderate improvement in emotional competence. However, *Coprococcus* levels predicted the response to inulin in terms of improved mood since the beneficial effect of inulin only occurred in participants with increased *Coprococcus* levels at baseline.

In another study Kavyani et al. wanted to assess co-supplementation of camelina sativa oil (CSO) and prebiotic as modulators of the gut microbiota on cardiometabolic risk factors and mental health in patients diagnosed with non-alcoholic fatty liver disease (NAFLD) and a BMI ≥ 25-30 kg/m^2 (Kavyani et al. 2021). The intervention group received 15% of the daily fat intake as CSO and 10 g/d resistant dextrin combined with a low-calorie diet (-500 kcal) for 12 weeks. After 12 weeks there was an observed improvement in mental health and stress as measured by general health questionnaire (GHQ) and depression, anxiety, and stress scale (DASS). The intervention group had a significant decrease in body weight and BMI compared with placebo, even though both groups were on a low-calorie diet. Co-supplementation of CSO and resistant dextrin decreased insulin concentration, HOMA-IR (a measurement of insulin resistance), hs-CRP, cortisol, malondialdehyde (MDA) and lipopolysaccharide (LPS) endotoxin and increased total antioxidant capacity (TAC) and superoxide dismutase (SOD) ($p<0.05$). Supplementation of CSO and resistant dextrin for 12 weeks improved glucose homeostasic indices, metabolic endotoxemia, oxidative stress, inflammation, and mental health.

Farhangi et al. studied the effect of 10 g/d Nutriose®06 FM from Maize, a resistant dextrin with prebiotic properties, in women with type 2 diabetes (T2DM) between 30-65 years old on mental health parameters (Farhangi et al. 2017). The aim of the study was to examine the effect of resistant dextrin on immune-mediated inflammation and HPA-axis in women with T2DM over a period of eight weeks. There was a significant improvement in GHQ and DASS scores. Furthermore, the intervention led to a reduction in body weight, BMI, and fasting insulin concentration. The intervention group also had a significant decrease in dietary composition including energy, carbohydrate, and total fat, compared with placebo and baseline. After eight weeks there was a significant decrease in levels of cortisol, kynurenine/tryptophan (KYN/TRP) ratio, and LPS. No significant differences were observed for TRP, adrenocorticotropic hormone (ACTH) or KYN. The only immune system response that changed during the study period was CD8 which significantly increased in the intervention group. Moreover, there was a significant decrease in levels of IFNγ and IFNγ/IL10 ratio and an increase in levels of IL-10 in the

intervention group compared with placebo. No significant differences were observed for IL-4 and IL-12.

Saleh-Ghadimi et al. also assessed the effect of 10 g/d resistant dextrin in females with T2DM for a period of eight weeks (Saleh-Ghadimi et al. 2022). However, they examined the effect on sleep and quality of life in females with both T2DM and a BMI ≥ 30 kg/m^2. Supplementation of resistant dextrin demonstrated an improvement in total score of sleep and its components except sleep disturbance. The final mean (SD) short form 36 (SF-36), which measures quality of life, showed a significantly higher improvement in the prebiotic group compared with placebo. Among the SF-36 categories, participants reported higher improvement in general health, vitality, and mental health parameters. Assessment of dietary intake revealed a significantly lower intake of energy, refined sugar, and total fat only in the intervention group. There was also an observed improvement in cortisol in the intervention group. Prebiotic administration led to a significant decrease in glycosylated hemoglobin (HbA1c), compared with placebo, however, fasting blood sugar (FBS) were not significant. For the inflammatory biomarkers, IL-18, IL-6, and tumor necrosis factor alpha (TNFα) decreased significantly, and IL-10 increased significantly in the prebiotic group compared with the placebo group at the end of the study. For the biomarkers of the HPA axis function, there was a significant decrease in levels of endotoxin, KYN/TRP ratio, and ACTH in the prebiotic group. No significant differences were found for TRP and KYN. A linear regression analysis revealed that changes in the metabolic endotoxemia, proinflammatory cytokines, cortisol, and KYN/TRP ratio parameters were predictors of changes in The Pittsburgh Sleep Quality Index (PSQI) score.

In a study not related to metabolic disorders patients ≥ 20 years old diagnosed with major depressive disorder received 7 g/d of the prebiotic 4G β-D-Galactosucrose (3.2 g Lactosucrose, LS) containing syrup, known to improve microbiome diversity due to fermentation by *Bifidobacterium* (Tarutania et al. 2022). To what extent LS can improve symptoms of depression was not known. The aim of the study was therefore to assess if administration of LS, taken once a day for 24 weeks, could influence depression. Depression was measured by Montgomery Asberg Depression Rating Scale (MADR), global self-efficacy scale (GSES), quality of life (QOL), and 16-item quick inventory of depressive symptomatology (QIDS). The results showed no significant scores either in MADRS, QOL, or QIDS score. The GSES score showed a tendency to improve in the intervention group compared with placebo (p=0.091), but the effect was not significant. Administration of 4G-β-D-Galactosucrose (LS) showed no difference in the prevalence of *Bifidobacterium* (p=0.710) nor a difference in microbiome

diversity between visit zero and six. Another study assessed whether supplementation of 10 g/day inulin together with a 25% calorie restricted diet for eight weeks in obese women in the age 20-50 years old diagnosed with major depressive disorder influenced mental health (Vaghef-Mehrabany et al. 2021). The study discovered no effect of the prebiotic supplementation on depression, but an effect of weight loss was observed. Participants with a weight loss ≥ 1.9 kg showed a significant reduction in Hamilton depression rating scale (HDRS) score ($p=0.013$) and a decrease in Beck depression inventory-II (BDI-II) scores ($p=0.060$) as well as a higher decrease in total cholesterol (TC) and fat mass ($p<0.05$).

Yet another study looked at young individuals, this time a group identified as academically stressed students supplemented with GOS (0, 2.5 or 5 g/d for eight weeks) (Hughes et al. 2011). Acute psychological stress followed by academic exams had a negative effect on gastrointestinal and immune function. The aim of the study was therefore to assess whether GOS intake could have a beneficial effect on the gastrointestinal function and percentage of days with cold or flu. It was observed that stress was positively related to the five gastrointestinal symptom score categories for all groups and was highly significant ($p<0.01$) for diarrhea, indigestion, reflux syndromes, and abdominal pain, but not constipation. Across all levels of stress, GI-symptom scores were significantly lower with GOS supplementation for diarrhea, constipation, abdominal pain, and indigestion, but not reflux syndromes. Students with a healthy weight who received 5.0 g GOS reported a 40 % reduction in the probability of having a sick day compared with those receiving 0 and 2.5 g GOS. For obese students, those receiving 2.5 g GOS experienced a lower percentage of sick days than those receiving 0 or 5 g GOS. The study found that acute psychological stress in students was directly related to GI-symptoms and cold/flu. Administration of GOS relieved these symptoms. One of the few studies carried out in children aged 7-9 years old assessed the effect of supplementation with β-GOS over a period of 12 weeks (Captião et al. 2019). The primary objective of the study was to investigate prebiotics influence on reading and cognitive abilities in primary school children. As a second aim, the study examined prebiotics effect on sleep, mood, and anxiety since poor reading abilities can impact both cognition and behavior. β-GOS had no significant effect on anxiety and mood as measured by the State-Trait Anxiety Inventory for Children (STAIC) and the Children Mood and Feeling Questionnaire - child short version (SMFO). Furthermore, there were no observed effects on sleep as measured by the Child Sleep Habits Questionnaire (CSHQ) and sleep diaries, and objectively measured using actigraphy (MotionWatch8). Behavioral

problems were also examined by the Conners´ Scale for both parents (CPRS-S) and teacher (CTRS-L) with no observed effect.

The final study on prebiotics examined healthy females between 18-40 years old receiving 12.5 g/d of Litesse®Ultra, a polydextrose (PDX) powder, for four weeks (Berding et al. 2020). Administration of PDX did not lead to an improvement in levels of anxiety (HADS-A), depression (HADS-D, BDI-II), perceived stress (PSS), or psychopathological symptoms (SCL-90-R global severity index). Supplementation with PDX in healthy females led to a significant increase in the genus *Ruminiclostridium.* No significant differences were observed for α- or ß-diversity (p=1.0 and p=0.996, respectively). Furthermore, the study found that acute stress significantly increased the expression of the adhesion receptor CD26L on classical monocytes in the placebo group compared with the PDX group (p=0.005). Berding et al. found no difference in the participants inflammatory profiles of plasma samples or in the TLR4 stimulated whole bloods. In the TLR5 stimulated bloods, there was a trend for lower concentrations of IFNγ (p=0.087) and IL-2 (p=0.074) after PDX supplementation compared with placebo. No differences were observed for cortisol levels.

To summarize, the four studies examining the effect of prebiotics in participants with a metabolic disorder observed a beneficial effect of prebiotic administration. The study who assessed the effect of inulin in obese subjects with the presence of at least one metabolic obesity related disorder demonstrated a moderate improvement in emotional competence (Leyrolle et al. 2021). The three other studies observed an improvement in general health and mental health parameters after administration of the prebiotic resistant dextrin (Kavyani et al. 2021, Farhangi et al. 2017, Saleh-Ghadimi et al. 2022). Furthermore, they demonstrated a beneficial effect on glucose homeostasic indices, metabolic endotoxemia, oxidative stress, and inflammation. In contrast to the two other studies, Saleh-Ghadimi et al. also observed an improvement in sleep quality after administration of resistant dextrin, a parameter not measured by the other studies. The beneficial effect of prebiotic intake on general health and mental health parameters was not observed in patients diagnosed with major depressive disorder (Tarutania et al. 2022, Vaghef-Mehrabany et al. 2021). However, Vagheh-Mehrabany et al. observed a significant reduction in depressive symptoms in participants with a weight loss ≥ 1.9 kg. There was also a tendency for an improvement in self-efficacy after supplementation of 7 g of 7 4G-β-D-Galactosucrose syrup (LS) for 24 weeks (Tarutania et al. 2022).

The three last studies examined the prebiotic effect in students, pre-school children and healthy females. Hughes et al. observed that acute psychological stress in students was directly related

to GI-symptoms and cold/flu, and that daily intake of GOS for eight weeks relieved these symptoms (Hughes et al. 2011). The study carried out in pre-school children did not find a significant effect of intake of β-GOS for 12 weeks on sleep, anxiety, behavioral problems, or mood (Captião et al. 2019). The final study carried out in healthy females found similar results as the previous study (Berding et al. 2020). Intake of the prebiotic PDX for four weeks did not lead to an improvement in anxiety, depression, perceived stress, or psychopathological symptoms. However, prebiotic intake led to a significant increase in the genus *Ruminiclostridium.* Out of the nine studies who assessed prebiotics influence on mental health parameters, five of the studies illustrated a beneficial effect, whereas four of the studies found no effect.

3.1.2 Prebiotics effect on cognition

Out of the studies who assessed the prebiotic effect on mental health, some also examined if prebiotic intake had an effect on cognition. The cognitive parameters assessed were flexibility (two studies), sustained attention, speed of processing, and verbal and executive functions. The prebiotics assessed were inulin, β-GOS, and PDX.

Leyrolle et al. studied inulin´s effect on cognition as well as on emotion and mood in obese participants (described in 3.1.1). For cognition, daily inulin supplementation significantly improved flexibility (decrease of Z-score and reaction time) (Leyrolle et al. 2021). Since mood and cognition can be related, they tested whether *Coprococcus* levels also influenced cognition. Baseline *Coprococcus* levels was not related to cognition as the high *Coprococcus* participants did not have an improved cognition. Supplementation of 16 g inulin for a period of 12 weeks have a moderate beneficial effect on cognitive flexibility in obese participants. Another study examined the effect of daily intake of β-GOS for 12 weeks in stable psychosis participants aged 18-60 years old (Kao et al. 2019). The aim of the study was to investigate the prebiotic effect on cognitive function, weight, and immune- and metabolic markers. A significant increase in the composite T-score (effect size) was observed after GOS administration, but not with placebo. The Brief Assessment of Cognition in Schizophrenia (BACS) were divided into two groups to explore which domains lead to the prebiotic-mediated improvement: verbal (verbal memory, verbal fluency) and executive (digit sequencing [measures verbal working memory], symbol coding, Tower of London) functions. There was an improvement in the executive, but not verbal, domains after supplementation with β-GOS ($p=0.045$). The overall effect in T-score of β-GOS was therefore driven by executive functions. However, it is important to state that there was only a slight improvement in the executive domains from baseline compared with

post-intervention (digit sequencing; p=0.423, symbol coding; p=0.127, Tower of London; p=0.200). The overall changes in T-scores from 0-24 weeks was only significant in those receiving β-GOS (p=0.040). There were no significant differences in mood, anthropometric measurements or serum levels of acetate, CRP, and IL-6 after supplementation with β-GOS. The study showed that intake of β-GOS significantly improves cognition in medicated participants with psychosis, possibly driven by improvement in executive functions.

A study carried out by Capitão et al. was one of few who reviewed the prebiotic effect on cognitive measurements in children (Captião et al. 2019). The main aim of the study was to examine if daily supplementation with β-GOS could influence reading and cognitive abilities in children. Reading improved significantly over time in both the intervention group and the placebo group, but there were no significant differences between the groups. The same was observed for the memory retrieval speed as measured by CogTrack^{TM} test battery. There was an increased reaction time in both treatment groups, but no significant difference between groups. The study found no effect of β-GOS on reading, working memory or cognition. Another objective of the study by Berding et al. was to examine if PDX could improve cognitive performance through manipulation of the gut microbiota (Berding et al. 2020). It was demonstrated that PDX supplementation led to an improvement in cognitive flexibility as recorded by a decrease in the number of errors made in the Intra- extra dimensional set shift (IED). The IED task is designed to test behavioral set-shifting abilities. Furthermore, PDX had a beneficial effect on sustained attention as observed through a higher number of correct responses and rejections in the Rapid Visual Information Processing (RVP) task. The RVP task measures sustained attention and speed of processing. For the IED task, the participants who received PDX had less errors (p=0.001) and completed the stages using a lower number of trials (p<0.001) compared with placebo. For the RVP task, there was an increase in number of correct responses (p=0.003) and total correct rejections (p=0.001) in the PDX group. The study found an improvement in cognitive performance in healthy women after treatment with PDX.

Out of the four studies that examined prebiotics effect on cognitive parameters, three of the studies found a beneficial effect. Leyrolle et al. found that daily intake of inulin led to an improved cognitive flexibility in obese participants (Leyrolle et al. 2021). Furthermore, the study did not find a correlation between cognitive parameters and abundance of *Coprococcus.* For the participants diagnosed with psychosis there was a significant improvement in cognitive function, possibly driven by improvement in executive functions, after intake of β-GOS for three months (Kao et al. 2019). The study carried out by Captião et al. also reviewd the effect

of β-GOS intake on cognitive measurements (Captião et al. 2019). However, the prebiotic was administered by pre-school children with illiteracy and found no effect on reading, working memory or cognition. The last study found that intake of PDX for four weeks led to an improvement in cognitive flexibility and sustained attention in healthy females (Berding et al. 2020). The findings of these studies indicates that intake of prebiotics may have a beneficial effect on cognitive flexibility, sustained attention, and executive functions. However, intake of prebiotic did not seem to influence cognitive measurements in children.

Table 3. The most important characteristics and results of the included studies on prebiotics.

Study	Design	Outcome	Effect of intervention
Leyrolle et al. 2021	RCT, single-blinded 106 men and women with a BMI > 30kg^2 and metabolic obesity-related disorder 18-65 y 12-week daily intake of 16 g inulin or 16 g placebo (maltodextrin)	PANAS Emotional competence (PEC TOT) SPANE NE, -PE Flexibility and working-memory (Z-score) Fasting glycaemia, HbA1c, liver enzymes, and lipids Cytokine levels (IL-1B, IL-8, IL-12p70, IL17a, MCP1, TNFα, IFNγ) Gut microbiota composition	Tendency of improved emotional competence Decreased negative emotion and improved cognitive flexibility Positive responders had an increased IL-8, *Bifidobacterium* and *Haemophilus* No differences in other cytokine levels Decrease in metabolic pathways in positive responders while no changes or an increase in negative responders Participants with high baseline *Coprococcus* had an improvement in emotional competence and mood Participants with low baseline *Coprococcus* had an improvement in flexibility and working-memory
Farhangi et al. 2017	RCT, triple-blinded 55 females with type 2 diabetes and BMI > 25kg/m^2 30-65 y Eight-week daily intake of 10 g resistant dextrin or 10 g placebo (maltodextrin)	Fasting insulin Cortisol, KYN/TRP ratio, LPS, GHQ, and DASS TRP, ACTH, and KYN Immune system response IFNγ, IL10, IFNγ /IL10	Reduction in fasting insulin Improved cortisol, KYN/TRP ratio, LPS, GHQ, and DASS No sig. differences in TRP, ACTH and KYN Improved CD8, no other sig. differences in immune system response Improvement in IFNγ, IL-10, and IFNγ /IL-10 ratio
Saleh-Ghadimi et al. 2022	RCT, double-blinded 63 obese females with type 2 diabetes 30-65 y Eight-week daily intake of 10 g resistant dextrin or 10 g placebo (maltodextrin)	PSQI SF-36 HbA1c Fasting blood sugar IL-18, IL-6, IL-10, TNFα KYN, TRP, KYN/TRP ratio, cortisol, ACTH	Improved PSQI Improved SF-36, higher improvement in general health, vitality, and mental health Reduced HbA1c No sig. differences in fasting blood sugar Decreased IL-8, IL-6 and TNFα Increased IL-10 No sig. differences in KYN and TRP Reduced endotoxin, KYN/TRP ratio, cortisol, and ACTH
Vaghef-Mehrabany et al. 2021	RCT, double-blinded 45 non-menopausal obese women diagnosed with major depressive disorder 20-50 y Eight-week daily intake of 10 g inulin or 10 g placebo (maltodextrin)	Hamilton depression rating scale (HDRS) Beck depression inventory (BDI-II) Weight, BMI, waist, and hip circumference, fat mass SBP, DBP, resting metabolic rate (RMR) FBS, insulin, HOMA-IR, TC, TG, LDL-C, HDL-C	Reduction in HDRS and BDI-II in patients who reached a weight loss over 1.9 kg regardless of intervention or control Decrease in weight, BMI, waist and hip circumference, and SBP in both groups Reduced TC and HDL-C No differences in other glycemia and lipid markers

Tarutania et al. 2022	RCT, double-blinded 20 men and women with major depressive disorder 36 -72 y 24-week daily intake of 7 g LS or 7 g placebo (syrup)	Depressive symptoms (MADRS) Self-efficacy (GSES) Quality of life (WHO/QOL) Prevalence of *Bifidobacterium*	No differences in depressive symptoms or quality of live Tendency of improved self-efficacy No difference in prevalence of *Bifidobacterium*
Kao et al. 2019	RCT, double-blinded 39 men and women with psychosis 18-60 y 12-week daily intake of β-GOS or placebo	Executive (verbal working memory) Verbal (verbal memory) BACS (Brief Assessment of Cognition in Schizophrenia) Mood Serum levels of acetate, CRP, and IL-6 Weight and BMI	Improved executive domains No sig. effect on verbal domains Improvement of BACS No sig. differences in mood, serum levels of acetate, weight, BMI, CRP, and IL-6.
Kavyani et al. 2021	RCT, double-blinded 36 men and women with NAFLD and overweight/obese 20-50 y 12-week daily intake of 15% of daily fat intake replaced with *Camelina sativa* oil (CSO) + 10g resistant dextrin or placebo (CSO and maltodextrin) and low-calorie diet (-500 kcal) in both groups	Fasting insulin HOMA-IR (homeostatic model assessment for insulin resistance) Hs-CRP LPS endotoxin FPG (fasting plasma glucose) Cortisol GHQ (general health questionnaire) DASS (depression, anxiety, and stress scale) TAC (total antioxidant capacity), GSH-Px (glutathione peroxidase), SOD (superoxide dismutase), uric acid, catalase, MDA, and 8-iso-PGF2a (8-iso-prostalglandin F2a)	Improved cortisol, general health and depression, anxiety, and stress Fasting insulin concentration, HOMA-IR, hs-CRP, and LPS endotoxin decreased significantly Decreases in FPG were non-significant. Decreased MDA, TAC, and SOD No significant differences in GSH-Px, uric acid, catalase, and 8-iso-PGFD2a Significant differences in TAC, SOD, GSH-Px, 8-iso-PGFa and MDA
Hughes et al. 2011	RCT, double-blinded 419 students who experienced at least one cold in the past year ≥ 18 y Eight-week daily intake of 5 g GOS or 2.5 g GOS + 2.5 g placebo (Baker's sugar (sucrose)) or 5 g placebo	Stress SI score Sick days GI symptoms	Stress was positively related to SI score Lower SI score across all levels of stress (2.5 g GOS) Lower SI score at lower levels of daily stress (5 g GOS) 40 % reduction in probability of having sick days in students with healthy weight (5 g GOS) Lower percentage of sick days in overweight and obese students; better improvement in those receiving 2.5 g than those receiving 5 g. Stress was positively related to GI symptoms Improved GI symptom scores
Capitão et al. 2019	RCT, double-blinded Parallell 25 male and female with below average literacy skills 7-9 y 12-week daily intake of β-GOS or placebo (maltodextrin)	Reading Sleep and immobile minutes Anxiety (STAIC) Mood (SMFQ) Behavioral problems (CPRS-L) Salivary cortisol concentration	No group difference on reading, sleep, cortisol levels or other parameters

Berding et al. 2022	RCT, double-blinded 18 healthy females 18-40 y Four-week daily intake of 12.5 g polydextrose or 12.5 g placebo (maltodextrin)	IED (cognitive flexibility) RVP (sustained attention) Diversity of gut microbiota CD62L Cortisol HADS-A, HADS-D, BDI-II, PSS, and SCL-90-R global severity index	Improved cognitive flexibility and sustained attention Increased *Ruminiclostridium* 5 Increase in CD26L by acute stress in control No sig. difference in cortisol, HADS-A, HADS-D, BDI-II, PSS, or SCL-90-R

3.2 Synbiotics effect on the brain

3.2.1 Synbiotics effect on mental health and cognition

A variety of studies have viewed the effect of synbiotics on mental health and cognition. The different combinations of pre- and probiotics used in these studies were: (1) probiotic (*L. acidophilus, L. casei, and B. bifidum*) + prebiotic (inulin) (2) probiotic (*L. paracasei, L. rhamosus, L. acidophilus, and B. lactis)* + prebiotic (FOS), (3) probiotic (*P. pentosaceus, L. casei,* and *L. plantarum*) + prebiotic (β-glucan, pectin, inulin, and RS), (4) probiotic (*L. rhamnosus*) + prebiotic (inulin), (5) probiotic (*B. lactis, L. rhamnosus, B. longum*) + prebiotic (FOS), and (6) probiotic (*L. acidophilus, B. bifidum, B. lactis)* + prebiotic (FOS, GOS and inulin). One study also included BCAA in addition to synbiotcs, the intervention group received probiotic (*L. paracasei, L. plantarum, L. mesenteroides, P. pentosaceus*) + prebiotic (2.5 g oat bran, 2.5 pectin, 2.5 g RS, 2.5 g crystalline starch) + BCAA (2.325 g leucine, 1.875 g isoleucine, 1.8 g valine).

Hadi et al. studied the effect on synbiotics in participants between the age 20-50 years with a BMI greater than 25 and less than 35 kg/m^2 (Hadi et al. 2019). The objective of the study was to examine the effect of daily synbiotic supplementation on anthropometric indices, glycemic and lipid profile, blood pressure, and psychological status of adults with overweight or obesity. The intervention group received one 500 mg capsule of synbiotic which contained *L. acidophilus, L. casei* and *B. bifidum* (2 x 10^9 CFU/g each) plus 0.8 g inulin daily for eight weeks. Administration of the synbiotic led to a significant improvement in stress ($p<0.001$), anxiety ($p=0.03$), and depression ($p=0.03$) as measured with DASS-21 compared with placebo. Furthermore, the intervention led to a reduction in triglycerides (TG), TC, LDL, and body weight ($P<0.05$). There were no observed differences in high density lipoprotein (HDL), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), and fasting insulin concentrations, as well as BMI and weight circumference (WC) ($P>0.05$).

Haghighat et al included 75 clinically stable hemodialysis (HD) patients aged 30-65 years with end-stage renal disease (ESRD) (Haghighat et al. 2021). The study aimed to examine the effects

of daily probiotic and synbiotic supplementation on depression and anxiety symptoms, as well as levels of BDNF. The participants were divided into three groups who received: (1) synbiotic supplement which contained the probiotics *L. acidophilus, B. bifidum, B. lactis, B. longum* (2.7 x 10^7 CFU/g each) per 5 g sachets and 15 g of prebiotics which contained 5 g FOS, 5 g GOS, and 5 g inulin per three 5 g sachets, (2) probiotic supplement with the same probiotics as in the synbiotic supplement, and (3) placebo which contained 15 g maltodextrin per three 5 g sachets. The intervention lasted for three months. 43 (66%) of the patients were diabetic and 49 (75%) were hypertensive. Bonferri post hoc test and paired t-test found a significant decrease in HADS-DEP score in the synbiotic group compared with placebo (p=0.004, p=0.009, respectively). In addition, paired t-test revealed a significant decrease in HADS-ANX score (p=0.047) in the synbiotic group and a significant decrease in HADS-DEP score (p=0.041) in the probiotic group. Bonferri post hoc and paired t-test also demonstrated a significant increase in serum BDNF level after administration of synbiotics (p=0.005, p=0.018, respectively) compared with placebo. The difference was also significant compared with the probiotic group (p=0.049). Based on HADS scores the patients were divided into two groups of non-depressed patients (n=26) and patients with depressive symptoms (n=49). In the non-depressed group, there was no significant difference in serum BDNF level, HADS-DEP or HADS-ANX scores over the 12 weeks regardless of supplementation. In the depressed group, paired t-test and one-way ANOVA revealed a significant reduction in HADS-DEP score in the synbiotic group ($p < .001$ for both t-test and ANOVA) and the probiotic group (p=0.004, p=0.011, respectively) after intervention. There was a similar reduction in HADS-DEP score in the synbiotic and probiotic group as measured with a Bonferri post hoc test ($p < 0.001$, p=0.037, respectively). Moreover, there was a reduction in HADS-ANX in the synbiotic and probiotic group as measured with paired t-test, but only the synbiotic group was statistically significant (p=0.030).

In the depressive group, paired t-test revealed an increase in serum BDNF levels in the synbiotic group (p=0.002) (Haghighat et al. 2021). The serum BDNF levels was also significantly different between the supplementation groups at the end of the study (p<0.001). Bonferroni post hoc test revealed that serum BDNF significantly increased in the synbiotic group compared with placebo (p=0.001) and probiotic group (p=0.002). Synbiotic supplementation led to a greater improvement in HADS depression score and serum level of BDNF protein compared with probiotic in ESRD patients undergoing HD. A decrease in HADS depression score was observed in the synbiotic group compared to the placebo group. Furthermore, the synbiotic supplementation resulted in an increased serum level of BDNF and decreased the HADS scores

compared to the probiotic in the HD patients with depression symptoms, but not in the non-depressed HD patients. For the changes in microbial diversity there was an increase in fecal bifidobacteria and lactobacili colonies and decrease in coliform colonies at week 12 in the synbiotic and probiotic group compared with baseline ($p<0.001$) and placebo group ($p<0.001$).

Moludi et al. carried out a study on participants between 18-85 years with cardiovascular disease (CAD) (Moludi et al. 2021). The study aimed to explore the anti-inflammatory and anti-depressant effects of *L. Rhamnosus,* alone or in combination with inulin, in patients with CAD. The participants were assigned into four groups: (1) prebiotic group who received 15 g inulin per day, (2) probiotic group who received 1.9×10^9 CFU of *L. rhamnosus* per day, (3) co-supplemented group who received inulin and *L. rhamnosus,* and (4) placebo who received maltodextrin. The intervention lasted for eight weeks. BDI-11 scores were measured for all participants to categorize them into two groups: depressed or non-depressed. For BDI and STAI-trait scores there was a significant decrease in the probiotic group ($p=0.001$, $p=0.006$, respectively) compared with baseline. Co-supplementation of pro- and prebiotic amplified the improvement of physiological outcomes more than either pro- or prebiotic alone. Probiotic + prebiotic significantly reduced BDI ($p=0.001$), STAI-state ($p=0.021$), and STAI-trait ($p=0.020$) compared with placebo. There were no significant differences in MacNew scores. The study found no significant differences in fasting blood sugar (FBS), total cholesterol (TC), LDL, TG, HDL, diastolic blood pressure (DBP), and systolic blood pressure (SBP) within groups. The group who received the co-supplementation had a slight reduction in SBP ($p=0.473$), TG ($p=0.269$) and cholesterol ($p=0.358$) compared with placebo. Furthermore, there was a significant reduction in LPS and TNFα ($p<0.05$) in the probiotic group compared with placebo. In the co-supplemented group, there was a significant reduction in serum hs-CRP, LPS, and TNFα ($p<0.05$) compared with placebo. No significant differences were observed for IL-10 concentrations. These findings indicate that co-administration of inulin and *L.* in CAD patients has a beneficial effect on inflammation, depression, and anxiety.

Another study assessed the effect of synbiotics administered daily over a period of nine weeks in children and adults between the age 5-55 years diagnosed with attention deficit hyperactivity disorder (ADHD) (Skott et al. 2020). The Synbiotic 2000 contained 4×10^{11} CFU per dose of three lactic acid bacteria: *P. petosaceus, L. casei, L. plantarum* and 2.5 g each of the fermentable fibers β-glucan, inulin, pectin, and RS. The study aimed to investigate effects of synbiotics on psychiatric symptoms in children and adults without an autism diagnosis. In children there was a trend in reduction of autism symptoms as measured by Social Communication Questionnaire

(SCQ) score after synbiotic supplementation. Furthermore, synbiotic supplementation significantly reduced the restricted, repetitive, and stereotyped behaviors compared with placebo. In adults there were no observed effects of synbiotic on autism symptoms as measured by Autism Spectrum Quotient (AQ) score. The baseline sVCAM-1 levels in ADHD patients were elevated compared with healthy controls. Elevated sVCAM-1 levels are common in inflammatory disorders such as IBD and metabolic disorders. The results showed that the synbiotic-specific improvement in total autism score was driven by children with elevated sVCAM-1 levels. In adults with elevated sVCAM-1 levels, synbiotics only led to a minor improvement in total autism score compared with placebo. The synbiotic improvement in total ADHD symptoms and the restricted, repetitive, and stereotyped behavior was driven by the children without ADHD medication. For adults there was an observed synbiotic improvement in difficulties in engaging in goal-directed behavior. For those with elevated sVCAM-1 levels at baseline, there was a synbiotic-specific improvement in emotion regulation total scale and the subdomains clarity, goals, strategies, and nonacceptance. There was an observed reduction of autism symptoms in children and an improvement of emotion regulation in adults with ADHD in those with elevated plasma sVCAM-1 levels.

A study on synbiotics aimed to investigate the effect of a synbiotic supplement on symptoms of brain disorders and inflammation in pre-frail elderly in the age 65-90 years (Louzada et al. 2018). The synbiotic group received two daily doses (6 + 6 g) of the prebiotic FOS (6 g) plus 10^9 - 10^8 CFU each of the probiotics *L. paracasei, L. rhamnosus, L. acidophilus, and B. lactis.* The study was conducted in elderly aged 65 to 90 years, and the intervention lasted for 24 weeks. At the end of the study there was an observed increase in Geriatric-Depression Scale-15 (GDS-15), which indicates a worsening of depressive symptoms. For Mini-mental status examination (MMSE) there was an observed increase, which indicates an improvement in cognitive status. Furthermore, IL-6 were increased in both groups, while %fat, TNFα, and diamine-oxidase (DAO) were reduced in both groups. Intestinal fatty-acid binding protein (IFABP) did not change in any group. No significant differences between these parameters were found between the intervention and control group. The synbiotic group experienced an increase in IL-10, whereas a decrease in LPS was observed in the placebo group from baseline to the end of the study ($p=0.05$). The study also did a multiple regression analysis to investigate associations between the variables. The only variables with statistically significant correlation with the final GDS-15 were the baseline GDS-15 ($p=0.03$), which demonstrated a positive association, and DAO ($p=0.01$) with a negative association. For final MMSE, there was a

significant correlation with baseline MMSE ($p<0.001$) and IL-10 ($p=0.003$), both with positive associations. These findings predict that intestinal permeability biomarkers and inflammatory cytokines may influence GDS-15 and MMSE.

The only study who compared athletes and sedentary people were Quero et al. (Quero et al. 2021). The aim of the study was to investigate the possible immunophysiological effects of daily intake of a supplement containing a mixture of probiotic strains, as well as the prebiotic FOS over a period of four weeks. 27 male participants, 13 professional soccer players and 14 sedentary students with low level of physical activity (≤150 min/week) were included. The intervention group received the synbiotic Gasteel Plus containing the probiotics: *B. lactis, L. rhamnosus, B. longum,* and 200 mg of the prebiotic FOS. Each stick of Gasteel Plus (300 mg) included lyophilized bacteria powder, equivalent to ≥ 1 x 10^9 CFU and 1.5 mg zinc, 8.35 µg selenium, 0.75 µg vitamin, and maltodextrin. The placebo sticks contained 300 mg maltodextrin. The synbiotic supplementation led to a significant improvement in the perceived general health in athletes ($p<0.05$) compared with baseline as determined by the SF-36 questionnaire. However, there were no differences in perceived sleep quality, state anxiety, or fatigue. There was an observed decrease in levels of perceived stress ($p<0.01$) and anxiety ($p<0.05$) in the athlete group who received the synbiotic. Synbiotic supplementation in both groups resulted in a reduction in perceived depression levels ($p<0.05$). There was no significant effect on metabolic profile, and no significant differences for IL-10 and immunoglobulin A between groups. Training affected the response to the synbiotic intervention since it induced a significant increase in the dopamine concentrations only in athletes ($p<0.05$). There was an observed decrease in epinephrine levels in the sedentary groups administered with the synbiotic ($p<0.05$) compared to baseline. There was a significant decrease in the sedentary group ($p<0.05$) compared with baseline and a slight increase in the soccer players. There were no significant differences in cortisol or ACTH.

The only study who assessed the effect of synbiotic and branched chain amino acids (BCAA) in patients with hepatic encephalopathy (HE) was carried out by Vidot et al. (Vidot et al. 2019). HE is characterized by reduced hepatic ammonia clearance, which is accompanied by alterations in the gut microbiota. Pro- and prebiotics have been demonstrated to beneficially modulate the gut microbiota, whereas BCAAs are thought to have a role in the detoxification of ammonia. The primary aim of the study was therefore to investigate the effect of daily supplementation with synbiotics and/or BCAA on HE in patients treated with lactulose. They received daily intake of either Synbiotic 200 Forte and branched chain amino acids (BCAA),

Synbiotic 200 Forte and placebo for BCAA, placebo for Synbiotic Forte 2000 and BCAA, or placebo for both over a period of eight weeks. All participants were under ongoing treatment of 63 mL lactulose per day. The 10 g of Synbiotic 2000 Forte contained the 10 x 10^{11} each of the probiotics: *L. paracasei, L. plantarum, L. mesenteroides, and P. petosaceus,* and the four fibers: 2.5 g oat bran, 2.5 g pectin, 2.5 g RS, and 2.5 g inulin. The placebo for synbiotics contained 10 g crystalline starch. The daily BCAA supplement, Hepatamine, which contained 2.325 g leucine, 1.875 g isoleucine, 1.8 g valine, contributed with 180 kcals. The placebo for Hepatamine contained a mixture of orange flavored powdered drink base and glucose and provided 180 kcals. The study found no significant differences over time in levels of depression and stress as assessed by the DASS-21 across the treatment groups. However, there was weak statistical evidence of a greater decrease in anxiety at eight weeks compared with baseline in the synbiotics+BCAA group compared with placebo in the intention-to-treat (ITT) analysis (p=0.06). The per-protocol (PP) analysis also showed a weak statistically significant reduction after four weeks (p=0.055), and a statistically significant reduction at eight weeks (p=0.035).

Administration of synbiotic+BCAA in patients with HE led to an improvement in trail marking test (TMT) B, a measurement of how fast participants can alternate between numerical and alphabetical systems, at 8 weeks relative to the baseline placebo group (ITT: p=0.018; PP: p=0.017) (Vidot et al. 2019). This finding indicates an improvement in HE due to an improvement in cognitive ability. No significant differences were found for TMT A, a test where participants must join 25 number scattered across the page in the correct order, likely due to a learning effect. The Inhibitory Control Test (ICT) was used to measure correct target responses, lures, and weighted lures which examines working memory, learning capacity, and response inhibition. For correct target responses there was a greater decrease in performance from baseline to four weeks for those in the placebo group compared to the synbiotic group (ITT: p=0.007; PP: p=0.008). The study did not find that changes in performance over time differed across intervention group for lure responses (ITT: p=0.52; PP: p=0.57). For weighted lures there was a reduction at four (ITT: p=0.049; PP: p=0.023) and eight weeks (ITT: p=0.049; PP: p=0.023) for all groups compared with baseline in the placebo group. There was a statistically significant interaction between intervention and time (ITT: p=0.015; PP: p=0.05). For the ITT analysis, the interaction was driven by a greater change from baseline in the synbiotic and BCAA alone groups at both four weeks (synbiotic: p=0.015; BCAA: p=0.026) and eight weeks (synbiotic: p=0.037; BCAA: p=0.01) compared with placebo. The interaction in the PP analyses was likely driven by a greater increase in weighted lure responses between

baseline and four weeks follow-up in the synbiotic+BCAA group relative to the changes over time in the placebo group (p=0.029). The results demonstrated an improvement in cognitive performance after supplementation of synbiotic+BCAA.

The different studies that assessed synbiotics effect on mental health and cognition included a variety of different groups of participants. The first study was carried out in obese or overweight participants and found that intake of synbiotic for eight weeks led to a significant improvement in stress, anxiety, and depression (Hadi et al. 2019). Furthemore, synbiotic intake had a beneficial effect on TG, TC, LDL, and body weight. Synbiotic intervention for three months in patients with ESRD observed similar effects on mental health parameters (Haghighat et al. 2021). However, this study also investigated the alone effect of probiotic, as well as the effect of synbiotics. Synbiotic intervention led to a significant decrease in depression and anxiety, whereas probiotic intervention led to a significant decrease in depression. The study divided the participants into two groups of non-depressed patients and patients with depressive symptoms. Synbiotic and probiotic supplementation led to a significant decrease in depression in the depressed group, the effect was greater in the synbiotic group. For anxiety, the reduction was only significant after synbiotic supplementation. This effect was not observed in the non-depressed group. Furthermore, the study found a significant decrease in serum BDNF levels in the depressed group after synbiotic supplementation. The study also measured microbial diversity and found that supplementation of synbiotic and probiotic led to an increase in fecal bifidobacteria and lactobacili colonies and a decrease in coliform colonies.

The third study assessed both the effect of prebiotic and probiotic alone, as well as the effect of co-supplementation (Moludi et al. 2021). Like the previous study, the participants were divided into two groups of depressed and non-depressed, however, the study was carried out in participants diagnosed with CAD. There was a significant reduction in depression and anxiety in the probiotic group, however this effect was amplified by co-supplementation of pro- and prebiotic. Furthermore, co-supplementation led to a significant reduction in serum hs-CRP, LPS, and TNFα. A reduction in LPS and TNFα was also observed for the probiotic group. Only one study assessed the synbiotic effect on children and adults diagnosed with ADHD (Skott et al.2020). Synbiotic supplementation led to an improvement in total ADHD symptoms and the restricted, repetitive, and stereotyped behavior. The effect was driven by children without ADHD medication. Synbiotic supplementation in adults led to an improvement in difficulties engaging in goal-directed behavior. The study observed that the synbiotic-specific improvement in total autism score was driven by children with elevated sVCAM-1 levels. In

adults with elevated sVCAM-1 levels, synbiotics only led to a small improvement in total autism score. Louzada et al. assessed the effect of synbiotic supplementation for six months in pre-frail elderly. The study observed a worsening of depressive symptoms and an improvement in cognitive status after intervention. Furthermore, IL10 was increased in the synbiotic group, whereas LPS was decreased in the placebo group. A linear regression analysis revealed a correlation between intestinal permeability biomarkers and inflammatory cytokines and depression and cognition.

Synbiotic intake in athletes led to a significant improvement in perceived general health, perceived stress, and anxiety in athletes (Quero et al. 2021). There was an observed reduction in perceived depression levels in both athletes and sedentary students. Furthermore, the study found that training affected the response to the synbiotic intervention since a significant increase in dopamine concentrations only was observed in the athletes. The last study assessed the combined supplementation of synbiotic and BCAA in participants diagnosed with HE. The study found no significant differences in depression and stress over time across the treatment groups. However, there was a statistically significant reduction in anxiety after eight weeks in the synbiotics+BCAA group. The study also found an improvement in cognitive ability which corresponds with an improvement in HE.

Table 4. The most important characteristics and results of the included studies on synbiotics.

Study	Design	Outcome measures	Effect of intervention
Hadi et al. 2019	RCT, double-blinded 59 overweight or obese men and women 20-50 y Eight-week daily intake of probiotic ($2+10^9$ CFU *L. acidophilus, L. casei, and Bifidobacterium bifidum*) + prebiotic (0.8 g inulin) or placebo (starch)	TG, TC, LDL, and HDL SBP, DBP, FPG, and fasting insulin Body weight, BMI, and WC Stress, anxiety, and depression	Improved TG, TC, and LDL No sig. differences in HDL, SBP, DBP, FPG and fasting insulin Improved stress, anxiety, and depression Reduced body weight
Haghighat et al. 2021	RCT, double-blinded 75 men and women with ESRD Mean age 46.64 (10.69) y 12-week daily intake of probiotic (*L. acidophilus, B. bifidum, B. lactis, B. longum* (2.7×10^7 CFU/g each) per 5 g sachets) + prebiotic (5 g FOS, 5 g GOS, and 5 g inulin per three 5 g sachets), or probiotic, or prebiotic, or placebo (15 g maltodextrin per 5 g sachets)	Fecal bifidobacterial, lactobacilli and coliform HADS-DEP HADS-ANX Serum BDNF Gastrointestinal symptoms (GSRS)	Increased bifidobacteria and lactobacilli and decreased coliform in synbiotc and probiotic group Reduced depression and anxiety in synbiotic group Reduced depression in probiotic group Increased BDNF in patients with depressive symptoms in synbiotic group Greater improvement in depression, anxiety and BDNF in synbiotic group compared to probiotic group in depressed participants

Moludi et al. 2021	RCT, double-blinded 88 men and women with cardiovascular disease 18-85 y Eight-week daily intake of $1.9x10^9$ CFU probiotic (*L. rhamnosus)* and 15 g prebiotic (inulin) or prebiotic or probiotic or placebo (maltodextrin)	LPS TNFα Serum hs-CRP IL-10 BDI and STAI-trait and STAI-state MacNew	Improved serum hs-CRP, LPS and TNF-a after synbiotic treatment Improved LPS and TNF-a levels after probiotic treatment No sig. difference in IL-10 Improved BDI and STAI-trait after probiotic treatment Improvement in BDI, STAI-state and STAI-trait after synbiotic treatment No sig. effect for MacNew score Co-administration of pre- and probiotic had better effect than either alone
Skott et al. 2020	RCT, double-blinded 182 children and adults diagnosed with ADHD Mean age 12 y + 36 y Nine-week daily intake of probiotic ($4x10^{11}$ CFU *Pedicoccus pentosaceus, L. casei,* and *L. plantarum*) + prebiotic (2.5 g each of β-glucan, pectin, inulin, and RS) or similar amount of placebo (maltodextrin)	ADHD symptoms Autism symptoms (SCQ scale) Autism traits (AQ scores) Inattention Hyperactivity Functioning sVCAM-1 DERS-16 (emotion regulation)	Improved autism symptoms in children Improved autism traits in adults and those with elevated sVCAM-1 levels Improved difficulties in engaging in goal-directed behavior in adults Improved emotion regulation, clarity, goals, strategies and nonacceptance in adults with elevated sVCAM-1 Improvement in autistic symptoms were higher in children without ADHD medication Improved autism symptoms in children with elevated sVCAM-1
Louzada et al. 2018	RCT, double-blinded 49 pre-frail men and women 65-90 y Two daily doses (6+6 g) of probiotic (10^9-10^8 CFU *L. paracasei, L. rhamosus, L. acidophilus, and B. lactis)* + prebiotic FOS or placebo (6 + 6 g maltodextrin) for 24 weeks	IL-6, IL-10, and TNF-a LPS IFABP DAO GDS-15 (depression) MMSE (mental status)	Improved IL-10 No significant changes in IL-6, TNF-a, IFABP and DAO between groups Reduced LPS in control No sig. differences in GDS-15 or MMSE score
Quero et al. 2021	RCT, triple-blinded 27 males: 13 professional soccer players + 14 sedentary students 18-30 y Four-week daily intake of probiotic (≥$1x10^9$ CFU *B. lactis, L. rhamnosus, B. longum*) + 200 mg prebiotic (FOS) + 1.5 mg zinc, 8.25 μg selenium, 0.75 μg vitamin and maltodextrin or 300 mg placebo (maltodextrin)	Sleep efficiency and latency determined by accelerometry Perceived general health (SF-36) Perceived sleep quality, anxiety, depression, and fatigue IL-B, IL-10 and immunoglobulin A Dopamine and epinephrine concentration CRH section Cortisol and ACTH	Athlete group: Improved sleep efficiency and latency Improved perceived general health No difference in perceived sleep quality or stress Decrease in perceived stress and anxiety Increased dopamine Sedentary group: Increased IL-B Decreased epinephrine Reduced CRH secretion Both groups: Decrease in perceived depression No sig. differences in cortisol and ACTH No sig. differences in IL-10 and immunoglobulin A
Vidot et al. 2019	RCT,double-blinded 49 men and women diagnosed with HE Mean age 55.8 y Eight-week daily intake of probiotic (*L. paracasei, L. plantarum, Leuconostoc mesenteroides, P.*	TMT A and B Correct target response Performance Depression and stress (DASS-21)	Improved TMT B (synbiotic + BCAA) No difference in TMT A No sig. differences in correct target response Greater reduction in performance in control No sig. differences in DASS-21

	pentosaceus) + prebiotic (2.5 g oat bran, 2.5 pectin, 2.5 g RS, 2.5 g crystalline starch) + BCAA (2.325 g leucine, 1.875 g isoleucine, 1.8 g valine) or synbiotic and placebo or BCAA and placebo or placebo (10 g crystalline starch + orange flavored powdered drink)	Weak stat. evidence of reduction in anxiety (synbiotic+BCAA)

Table 5. Division of included studies based on type of prebiotic or synbiotic

Intervention		Studies (total: n = 17)
Supplementation with prebiotics	-Resistant dextrin	n=3
	-GOS / β-GOS	n=3
	-Polydextrose	n=1
	-Inulin	n=2
	-4G-β-D-Galactosucrose	n=1
Supplementation with synbiotics	-Probiotic (*L. acidophilus, L. casei, and B. bifidum*) + prebiotic (inulin)	n=1
	-Probiotic (*L. paracasei, L. rhamosus, L. acidophilus, and B. lactis)* + prebiotic (FOS)	n=1
	-Probiotic (*P. pentosaceus, L. casei,* and *L. plantarum*) + prebiotic (β-glucan, pectin, inulin, and RS)	n=1
	-Probiotic (*L. rhamnosus*) + prebiotic (inulin)	n=1
	-Probiotic (*B. lactis, L. rhamnosus, B. longum*) + prebiotic (FOS)	n=1
	-Probiotic (*L. acidophilus, B. bifidum, B. lactis)* + Prebiotic (FOS, GOS and inulin)	n=1
Supplementation with synbiotics + BCAA	-Probiotic (*L. paracasei, L. plantarum, L. mesenteroides, P. pentosaceus*) + prebiotic (2.5 g oat bran, 2.5 pectin, 2.5 g RS, 2.5 g crystalline starch) + BCAA (2.325 g leucine, 1.875 g isoleucine, 1.8 g valine)	n=1

4 Discussion

4.1 Quality of included studies

All the included studies are RCT's with the aim to investigate the effect of pre- or synbiotics in humans. RCT's are considered the most reliable methods to investigate the effect of an intervention or treatment in humans due to their potential to limit bias (Hariton et al. 2018). The randomization ensures random distribution of observed and unobserved characteristics that may influence the results. The included studies are also blinded. Blinding is important to avoid observer bias from the participants (single-blinded), participants and experimenters (double-blinded), or participants, experimenters, and researchers analyzing the data (triple-blinded).

The studies are carried out in a various number of participants (18-419), nine of the studies are carried out in groups of less than 50 participants, and only two in groups of 20 or less participants. To be able to answer the research question with a degree of certainty, an adequate number of participants is required. A study with a low number of participants will to a lesser extent be representable for the general population. The strength of the study will be greater with a larger group of participants as the results are more likely to be representable.

When studying the gut microbiota and supplementation with pre- or synbiotics, several factors can influence the results. The various studies assess different types and doses of pre- and synbiotics, have different lengths of intervention, and includes participants with various diseases or conditions. Diversity of the results may therefore be due to baseline levels of measured parameters, type, dose and duration of intervention, differences in ethnicity, pathologic state, as well as individual differences in gut microbiota, genotype, mental health, immune status, and cytokine status of the subjects. Diet is another factor that may have influenced the results. A number of the included studies also had dietary interventions which to some extent may influence the composition of the gut microbiota. Improved dietary habits may lead to an improved composition and as a result mask the effect of the supplement.

Different tools were used to measure mental health and cognitive parameters. For emotion, anxiety, depression, and stress the different tools used were PEC, SPANE, HDRS, BDI-II, MADRS, GSES, STAIC, SMFQ, DERS-16, GDS-15, MMSE, DASS-21 and HADS. PSQI were used to measure sleep quality, whereas SF-36, GHQ, QOL, and MacNew were used to measure quality of life. For cognition the different studies assessed cognitive parameters with the tools BACS, IED, RVP, and TMT. The various different tools used may also have influenced the diversity of the results and is a factor to consider when comparing the findings of the different studies.

4.2 Prebiotics and synbiotics effect on mental health

A majority of the studies found a beneficial effect of prebiotic or synbiotic administration on mental health. Out of the 10 studies examining prebiotics, all of them aimed to assess the effect on mental health, and a beneficial effect was observed in five of the studies. For synbiotics on the other hand, six out of the seven included studies aimed to assess the effect of synbiotics on mental health. All these studies observed a beneficial effect.

The thesis uncovered three studies who assessed the effect of intake of 10 g/d resistant dextrin in participants with metabolic disorder. Two of these was carried out in women diagnosed with T2DM, one of these with a BMI ≥ 30kg/m^2, and the last one in patients with a BMI ≥ 25-30kg/m^2 as well as being diagnosed with NAFLD (Farhangi et al. 2022, Saleh-Ghadimi et al. 2022, Kavyani et al. 2021). The three studies had similar findings and observed that prebiotic intake did have a beneficial effect on mental health. Eight-week daily administration of resistant dextrin led to a significant improvement in general health, depression, anxiety, and stress (Farhangi et al. 2022). Furthermore, it led to a significant improvement in total score of sleep, as well as an improvement in quality of life as measured by SF-36 (Saleh-Ghadimi et al. 2022). Among the SF-36 categories, participants reported a higher improvement in general health, vitality, and mental health parameters. The third study had a longer intervention of 12 weeks, but the results were in agreement with the two first studies (Kavyani et al. 2021). There was an improvement in general health and depression, anxiety, and stress after prebiotic intake. A difference from the other interventions with resistant dextrin is that the participants also received 15% of the daily fat intake as CSO and were put on a low-calorie diet (-500 kcal). However, two of the studies revealed a reduction in body weight, BMI, and insulin concentration, whereas all revealed a decrease in energy and macronutrient intake regardless of the low-calorie diet in the one study.

A reduction in body weight, BMI and fasting insulin was not observed in the study by Saleh-Ghadimi et al., however a reduction in HbA1c was discovered. It is difficult to predict whether a reduction in body weight, BMI, and energy and macronutrient intake in the intervention group influenced the results since the placebo group did not experience any changes in these measurements. The different studies also found an improvement in homeostasic indices, metabolic endotoxemia, oxidative stress, and inflammatory markers after intervention. Furthermore, a linear regression analysis revealed that changes in metabolic and inflammatory biomarkers, namely endotoxin and IL-6 and IL-8, respectively, were predictors of changes in quality of sleep (Saleh-Ghadimi et al. 2022). This may support the view that increased endotoxin levels have a negative effect on sleep and that resistant dextrin improves sleep by reducing levels of endotoxin. The same theory can be applied to the effect of IL-6 and IL-8. The correlation between biomarkers and SF-36 was not measured. However, Kavyani et al. also observed a decrease in endotoxin levels, whereas Farhangi et al. observed a beneficial effect on inflammatory cytokines after intervention (Kayvani et al. 2021, Farhangi et al. 2017). It may therefore seem like improvement in metabolic and inflammatory biomarkers as a result of

intake of resistant dextrin may influence mental health in people with metabolic disorders. Interestingly, two of the studies assessed general health and depression, anxiety, and stress with the GHQ and DASS.

Another study carried out in obese or overweight participants also demonstrated an improvement in stress, anxiety, and depression and a decrease in body weight after eight-week intervention (Hadi et al. 2019). However, the intervention group received a daily intake of synbiotics, containing 0.8 g inulin as well as different probiotic strains. Considering the small dose of prebiotics, it is likely that the probiotics also had an important role in the observed effects. Synbiotic supplementation also led to a reduction of cholesterol (TC and LDL) and triglycerides. The pre- and synbiotic effect on lipid profile is not fully understood, but the findings may be attributed to synbiotics effect on the gut microbiota. A second study also assessed the effect of inulin, but in the form of 16 g/d inulin for 12 weeks as well as a recommendation of intake of vegetables rich in inulin (Leyrolle et al. 2021). Similar to some of the mentioned studies, the participants had a BMI > 30kg/m^2 as well as the presence of at least one metabolic obesity-related disorder. This study also presented a beneficial effect of prebiotic administration on mental health but assessed slightly different measurements. Administration of inulin had a moderate beneficial effect on emotion regulation and a significant improvement in negative emotion. Furthermore, this was the first study to measure gut microbiota profile. The beneficial effect of inulin was only observed in the participants with an increase in *Coprococcus* levels at baseline. The observed effect of improved mood after inulin administration was therefore dependent on the individual composition of the gut microbiota. This supports the view that prebiotic effect on mental health is influenced by the baseline composition of the gut microbiota. Furthermore, there was a greater increase in *Bifidobacterium* and *Haemophilus,* as well as a significant increase in IL-8, and a higher decrease in DDP-IV, and subcutaneous fat mass in positive responders as compared with negative responders. These findings indicate a significant correlation between the positive score and *Bifidobacterium, Haemophilus,* IL-8, and subcutaneous fat mass. The observed prebiotic-influence on *Bifidobacterium* levels is supported by previous interventions in both humans and mice who found that prebiotics increased the relative abundance of *Bifidobacterium* (Birkeland et al. 2020, Burokas et al. 2016, Liu et al. 2017). The prebiotic intervention in mice also led to a reduction in depression and anxiety, similar to the findings from various of these studies.

Haghighat et al. also aimed to assess synbiotics effect on depression and anxiety (Haghighat et al. 2021). However, this study included participants with ESRD and compared the differences

in effect after intake of either synbiotic, probiotic or placebo for three months. This is the first study that used a combination of different prebiotics, namely FOS (5 g), GOS (5 g), and inulin (5 g) as well as different strains of probiotics. Like other mentioned findings, synbiotic supplementation led to a significant decrease in depression and anxiety, whereas probiotic supplementation alone led to a significant decrease only in depression. Levels of BDNF was significantly increased after synbiotic supplementation. None of the other studies measured BDNF, but the result is supported by a study in rats that observed an increase in brain BDNF expression after administration of prebiotics (Savignac et al. 2013). It can therefore be assumed that the increase in BDNF came as a result of prebiotic intake. Two of the studies on synbiotics decided to divide the participants into two groups of depressed and non-depressed based on depression scores (Haghighat et al. 2021, Moludi et al. 2021). However, it seems like only one of the study assessed the differences in effect of synbiotic supplementation between the two groups (Haghighat et al. 2021). The findings revealed that the reductions in depression and anxiety only occurred in the depressed group. Synbiotic supplementation significantly reduced depression and anxiety, whereas probiotic supplementation reduced depression. Moreover, the levels of BDNF significantly increased in the depressed group after synbiotic supplementation. This was not observed for the non-depressed group. Finally, the study demonstrated an increase in the fecal bifidobacteria and lactobacilli colonies and decrease in coliform colonies at week 12 in the synbiotic and probiotic group. As previously mentioned, an increase in *Bifidobacterium* is demonstrated in a number of previous studies on prebiotics as well as after intake of 16 g/d inulin (Leyrolle et al. 2021). This view is further supported by an observed increase in *Lactobacillus* after synbiotic supplementation in humans (Sergeev et al. 2020). The observed increase in lactobacilli is supported by a previous study who found an increase in *Lactobacillus* after administration of inulin in mice (Hoffman et al. 2019). The next study who divided the participants into groups of non-depressed and depressed, however was carried out in participants diagnosed with CAD (Moludi et al. 2021). Like a majority of the other studies, synbiotic supplementation for eight-weeks led to a decrease in depression and anxiety. The same effect was observed after probiotic administration, but the effect was amplified by the co-supplementation of the prebiotic 15 g/d inulin and probiotic *L. rhamnosus*. In contrary to the study by Hadi et al., there were no observed changes in cholesterol and triglycerides. However, the study did observe an improvement in metabolic and inflammatory biomarkers. One of the included the studies assessed the combined supplementation on synbiotics and BCAA in participants diagnosed with HE (Vidot et al. 2019). The observed reduction in levels of

depression and stress after eight-week intervention is likely due to the intake of synbiotics as the view is supported by a majority of these studies.

The two next studies assessed the prebiotic effect in participants with major depressive disorder (Vaghef-Mehrabany et al. 2022, Tarutania et al. 2022). In contrary to the mentioned findings, supplementation with prebiotics did not influence depressive symptoms or the levels of *Bifidobacterium*. However, an effect of weight loss was observed as participants with a weight loss greater than 1.9 kg had significantly lower depression score, but not after intake of 10 g/d inulin (Vaghef-Mehrabany et al. 2022). Intake of 7 g/d of the prebiotic LS for 24 weeks had no effect on body weight but it did, however, lead to a tendency of an improvement in self-efficacy. The result from these studies illustrates that treatment with prebiotics did not influence depressive symptoms in patients diagnosed with major depressive disorder. It may indicate that a higher dose of prebiotics is necessary to improve the assumed dysbiosis, and thereby the depressive symptoms in this patient group. The assumption that a higher dose might be necessary is supported by the observed increased levels of *Bifidobacterium* and the improvement in mental health after intake of 16 g/d prebiotics (Leyrolle et al. 2021). Other factors such as baseline gut microbiota profile, type of prebiotic and ethnicity may also have influenced the different effects on levels of *Bifidobacterium.* It is also important to consider the long duration (24 weeks) of the intervention, which may have made it more challenging to account for confounding factors and tolerance to the treatment may have been established (Tarutania et al. 2022).

In apparently healthy induviduals, it was greater difference in effect after pre- and synbiotic supplementation. The two studies carried out in students found a beneficial effect (Quero et al. 2021, Hughes et al. 2021), however, the effect was on different measurements. Quero et al. observed that synbiotic supplementation for four weeks led to a significant improvement in perceived general health, stress, and anxiety in athletes (Quero et al. 2021). For depression levels, there was an observed reduction in both the athlete and sedentary group after synbiotic supplementation. It was observed that training influenced the response to the synbiotic supplementation since it induced a significant increase in the dopamine concentrations only in athletes. The study found no differences in metabolic and inflammatory markers. Another study carried out in students observed that acute stress was directly related to GI-symptoms, cold/flu symptom intensity, and the percentage of days with cold/flu, and that GOS relieved these symptoms (Hughes et al. 2011). A dose of 2.5 g/d GOS had a greater beneficial effect on symptoms associated with abdominal pain and indigestion syndrome as compared with a dose

of 5 g/d GOS. Gastrointestinal symptoms may have been a contributing factor to the experienced stress, and this might be the reason why 5 g/d GOS lost its effect when levels of stress increased. Furthermore, the average percentage of days of cold/flu was reduced in healthy individuals supplemented with 5 g/d GOS. The same effect was not observed for underweight, overweight, or obese participants. It can therefore be assumed that differences in gut microbiota profile between these groups may have influenced the effect of GOS.

The next three studies did not find an improvement in mental health after intake og pre- and synbiotics (Berding et al. 2020, Louzada et al. 2018, Captião et al. 2019). Berding et al. investigated the effect of 12.5 g/d PDX in healthy females (Berding et al. 2020). In contrast to the previous mentioned studies, the study found no effect of prebiotic administration on levels of anxiety, depression, perceived stress, or psychopathological symptoms. However, the intervention did lead to a significant increase in the genus *Ruminiclostridium*. The intervention lasted for four weeks, which is a shorter duration than most of the other included studies. It may therefore be discussed if a longer intervention of prebiotics is necessary for a beneficial effect on mental health. However, a beneficial effect of synbiotic intake for four weeks was demonstrated (Quero et al. 2021) Another possible explanation may be that a majority of the other studies were carried out in participants with a disorder and their baseline gut microbiota profile and response to prebiotics may therefore have differed from healthy individuals. Furthermore, this is the only study assessing the effect of PDX, and it may be that PDX does not have the same beneficial effect on mental health. The study carried out by Louzada et al. in pre-frail elderly was the only study that observed a worsening of depressive symptoms after synbiotic supplementation (Louzada et al. 2018). In fact, it was the only included study that did not find a beneficial effect of administration of synbiotics on mental health. The intervention lasted for six months, and it is likely that the observed worsening was due to other uncontrolled factors. The pro- and prebiotics administered (*L. paracasei, L. rhamnosus, L. acidophilus, B. lactis* + FOS) was the same as those used in a number of the other included studies, which makes it unlikely that this was the reason for the findings. Furthermore, this was the only study that was carried out in pre-frail elderly which leads to the assumption that synbiotics may not lead to an improvement in mental health in this age group. However, the intervention did have a beneficial effect on inflammatory biomarkers.

For primary school children with below average literacy skills, intake of β-GOS for 12 weeks did not influence anxiety and mood, sleep, cortisol levels or other parameters (Captião et al. 2019). A possible explanation may be that the influence of gut bacteria on the brain is not fully

established in early life. Moreover, the study was carried out in apparently healthy participants. Skott et al. found that synbiotic supplementation in children and adults diagnosed with ADHD had a greater effect in children (Skott et al. 2020). Synbiotic supplementation in children led to a significant reduction in the restricted, repetitive, and stereotyped behaviors. The improvement was driven by children without ADHD medication. In adults, there were no observed effect of synbiotic supplementation on autism symptoms. However, there was an improvement in difficulties in engaging in goal-directed behavior after synbiotic supplementation. The study found that the observed effect of the synbiotic supplementation was driven by elevated sVCAM-1 levels in children with ADHD. In adults with elevated sVCAM-1 levels, there was only a minor improvement in total autism score after intervention, however, there was an observed improvement in emotion regulation. The different observed effects in the two studies carried out in children is likely due to the different groups of participants which makes it more challenging to compare the results (Captião et al. 2019, Skott et al. 2020). The first study was carried out in children with below literacy skills prescribed with prebiotics, whereas the other was carried out in children with ADHD prescribed with synbiotics.

4.3 Prebiotics and synbiotics effect on cognition

There was not as many of the studies assessing the effect on cognition as there were on mental health. From the findings of the included studies, it seems likely that prebiotic and synbiotic intake may have a beneficial effect on cognition in adults. Supplementation with the prebiotic's inulin, PDX, or β-GOS, and the synbiotics *L. paracasei, L. rhamnosus, L. acidophils, B. lactis* + FOS or *L. paracasei, L. plantarum, L. mesenteroides, P. pentosaceus* + oat bran, pectin, RS, and crystalline starch may lead to an improvement in cognitive measurements in adults. (Berding et al. 2020., Leyrolle et al. 2021, Kao et al. 2019, Louzada et al. 2018, Vidot et al. 2019)

Prebiotic supplementation in both patients with psychosis, healthy participants, and participants with a BMI > 30kg/m^2 as well as metabolic obesity-related disorder led to a beneficial effect on cognitive parameters (Berding et al. 2020, Leyrolle et al. 2021, Kao et al. 2019). There was no observed prebiotic-specific effect on mental health in healthy women, however there was an observed improvement in cognitive function (Berding et al. 2020). Administration of 12.5 g PDX for four weeks led to an improvement in cognitive flexibility, sustained attention, and speed of processing. These findings indicate that intake of PDX does not have a beneficial effect on mental health in healthy women but is does have a beneficial effect on cognitive

performance. Prebiotic supplementation in participants with a BMI > 30kg/m^2 and the presence of at least one metabolic obesity-related disorder experienced a moderate improvement in cognitive flexibility after administration of 16 g/d inulin for three months (Leyrolle et al. 2021). For patients diagnosed with psychosis, supplementation of β-GOS for three months led to an improvement in cognition, possibly driven by an improvement in executive functions (Kao et al. 2019). The intervention did not lead to a change in weight, BMI or metabolic- and inflammatory biomarkers. Supplementation of β-GOS in children, however, did not influence reading, working memory or cognition (Captião et al 2019). As previously stated, this may be due to age or a healthier gut microbiota.

In pre-frail elderly, synbiotic supplementation led to an improvement in cognitive status (Louzada et al. 2018). An increase in IL-10 was only observed in the synbiotic group, whereas a decrease in LPS was only observed in the placebo group. Final cognitive status was positively associated with baseline cognitive status and IL-10. This supports the view that intestinal permeability biomarkers and inflammatory cytokines may influence cognition. These findings are not in line with previous results that did not find an effect on cognitive status or LDL in elderly (Buigues et al. 2016). However, the participants received prebiotics and not synbiotics. The study by Kao et al. did not find a change in inflammatory biomarkers, which suggests that other mechanisms may also be responsible for the pre- and synbiotic effect on cognition (Kao et al. 2019). The final study assessed the effect of the combined treatment of synbiotic and BCAA in patients with HE (Vidot et al. 2019). There was an observed improvement in TMT B and ICT weighted lures after synbiotic +BCAA supplementation which may indicate an improvement in HE due to improved cognitive ability. No significant differences were observed for the TMT A between the intervention and placebo group, which is likely due to a learning effect. The study did not find an improvement in levels of HE when synbiotics and BCAA were taken separately, but combined supplementation led to a significant improvement in cognition, and therefore, executive functioning. Furthermore, the PP analysis revealed a statistically significant improvement in depression and stress after the combined supplementation. However, the significance of these findings can be discussed as the PP analysis is more subject to bias than the ITT analysis.

5 Conclusion

This systematic review aimed to assess how prebiotic and synbiotic supplementation affects mental health and cognitive measurements in humans. The results presented and discussed in this thesis, supported by a number of animal studies, demonstrates a promising beneficial effect of prebiotic and synbiotic supplementation on mental health, especially anxiety and depression, and cognitive measurements in humans. This effect, however, seems to be influenced by various factors such as individual differences in gut microbiota, pathological state, and type, dose and length of intervention, to mention a few.

Another important finding is that prebiotic and synbiotic supplementation may lead to an improvement in metabolic and inflammatory biomarkers. These findings propose that prebiotic or synbiotic supplementation may influence their effects on mental health parameters. Limited evidence was presented for the beneficial effect on the composition of the gut microbiota after prebiotic and synbiotic intake. Despite the limited evidence, it is safe to assume that the gut microbiota composition has an important role in the bidirectional communication between the brain and the gut.

The mechanisms behind prebiotics and synbiotics effect on the gut-brain-axis are not well established. Moreover, the type, dose, and duration of intervention necessary to provide a beneficial effect of prebiotic and synbiotic supplementation is still uncertain. Future research is therefore needed to investigate the potential use of prebiotics or synbiotics as therapeutical agents.

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