Final degree project
Double degree in Pharmacy and Human Nutrition and Dietetics

Could a supplementation with *Bifidobacterium infantis* in formula milk prevent ADHD development?

ANA MARINÉ SANTOS

Treball d'aprofundiment
Departament de Bioquímica i Fisiologia
Facultat de Farmàcia I Ciències de l'Alimentació
Univeristat de Barcelona

June 2025

Abbreviations:

- 2'-FL: 2'-fucosylatose
- 5-HT: Serotonin
- Ach: acetylcholine
- ADHD: Attention Deficit and Hyperactivity Disorder
- AEPap: Asociación Española de Pediatría de Atención Primaria
- ASD: autism spectrum disorder
- BBB: Blood–Brain Barrier
- CNS: Central Nervous System
- DA: Dopamine
- DAT: dopamine transporters
- DHA: docosahexaenoic acid
- DSM-II: second edition of Diagnostic and Statical Manual of Mental Disorders
- EFSA: European Food Safety Authority
- ENS: Enteric Nervous System
- EPA: Eicosapentaenoic acid
- Fe: Iron
- GABA: Gamma-aminobutyric acid
- Glu: Glutamate
- GSH: Glutathione
- HMO: Human Milk Oligosaccharides
- HPA axis: Hypothalamic-pituitary-adrenal axis
- IFN γ: Interferon gamma
- IL: Interleukins
- NE: norepinephrine
- NICE: National Institute for Health and Care Excellence
- PAPenRED: Red de Investigación en Pediatría de Atención Primaria.
- PET: Positron Emission Tomography
- ROS: Reactive Oxygen Species
- SCFA: Short Chain Fatty Acid
- SPECT: Single Photon Emission Computed Tomography
- Th2 cell: T helper 2 cells
- TNFα: Tumor Necrosis Factor alpha

Resum:

El trastorn de dèficit d'atenció i hiperactivitat (TDAH) és una condició que afecta a un elevat nombre de nens i adolescents arreu del món, amb una prevalença que continua augmentant. Tradicionalment, s'ha atribuït a factors genètics hereditaris, tot i que durant les últimes dècades s'han estudiat altres elements influents en el desenvolupament del trastorn. Aquest nou enfocament dona lloc a noves línies terapèutiques alternatives a les convencionals i genera interès per trobar tractaments més efectius de llarga durada.

El treball aprofundeix sobre l'etiologia del TDAH i, en particular, en l'impacte de la microbiota en l'aparició del trastorn. Cal destacar la presència de disbiosi com a característica comuna en molts dels factors de risc ambientals, fet que suggereix una possible implicació en l'aparició d'aquesta condició. Per entendre l'impacte de la microbiota intestinal en el neurodesenvolupament s'ha analitzat la seva relació amb el cervell a través de l'eix microbiota-intestí-cervell. Per últim, el treball examina la influència de la lactància materna en la formació de la microbiota del nadó i els seus efectes beneficiosos.

S'ha dut a terme una recerca sistemàtica a Pubmed, SpringerLink, Scopus, ScienceDirect i Nature, així com entrevistes presencials i telemàtiques amb especialistes de diferents àmbits: un gastroenteròleg, un pediatre, una psiquiatra infantil i un investigador sènior de Danone especialitzat en microbiota infantil i una representant de Nutribiótica.

Els resultats posen de manifest la importància de la neuroinflamació en el desenvolupament del TDAH. Aquesta s'origina com a conseqüència de la inflamació crònica, afavorida per la disbiosi, especialment en nens alimentats amb llet en fórmula. La suplementació amb *Bifidobacterium infantis* en nadons ha demostrat un efecte positiu en el desenvolupament del sistema immunitari a través de la modulació de la resposta inflamatòria. A més*, B. infantis* contribueix a la correcta formació de la microbiota intestinal.
En conclusió, malgrat la necessitat de més investigació, hi ha indicis que la suplementació de *B. infantis* en la llet en fórmula podria contribuir a prevenir el desenvolupament del TDAH.

Paraules clau: Trastorn d'hiperactivitat i dèficit d'atenció (TDAH), *Bifidobacterium infantis*, llet en fórmula, neuroinflamació, disbiosi

Abstract:

Attention deficit and hyperactivity disorder (ADHD) is a condition which affects a large number of children and adolescents worldwide, with a prevalence that continues to increase. Traditionally, it has been attributed to hereditary genetic factors, however, in the last few decades, other elements that influence the development of the disorder have been studied. This new approach has led to the exploration of alternative therapies beyond conventional ones and has generated growing interest in the search for more effective long-term treatments.

The thesis focuses on the aetiology of ADHD and, in particular, on the impact of the gut microbiota on the onset of the disorder. It is worth highlighting the presence of dysbiosis as a common feature in many environmental risk factors, which suggests a possible involvement in the development of this condition. To better understand the impact of the gut microbiota on neurodevelopment, its relationship with the brain has been analysed through the microbiota-gut-brain axis. Finally, the study explores the influence of breastfeeding on the formation of the infant microbiota and its associated benefits.

A systematic literature search was conducted using Pubmed, SpringerLink, Scopus, ScienceDirect and Nature. Additionally, in-person and online interviews were carried out with specialists from different fields: a gastroenterologist, a paediatrician, a child psychiatrist and a researcher from Danone specialised in infant microbiota and a sales-representative from Nutribiótica.

The findings underline the importance of neuroinflammation in the development of ADHD. This process is often the result of chronic inflammation, promoted by dysbiosis, especially in formula-fed children. Supplementation with *Bifidobacterium infantis* in infants has been shown to have a positive effect on the development of the immune system through modulation of the inflammatory response. Furthermore, *B. infantis* contributes to the healthy formation of the intestinal microbiota.

In conclusion, although further research is required, current evidence suggest that *B. infantis* supplementation in formula milk could contribute to preventing the development of ADHD.

Key words: Attention Deficit and Hyperactivity Disorder (ADHD), *Bifidobacterium infantis,* formula milk, neuroinflammation, dysbiosis

SGD (Sustainable Development Goals):

This research is a literature review on ADHD that explores the potential of *B. infantis* supplementation in infant formula to reduce the risk of developing ADHD, thus promoting health and wellbeing (SDG-3). Furthermore, this work has been conducted in an academic context using scientific literature, supporting quality education (SDG-4). The aim of the project is to study the addition of this probiotic in commercial formula milks. Since only a minority of commercial formula milk contain this probiotic, the research promotes innovation in food industry (SDG-9). Finally, the optimisation of resources and efficient formulations to ensure the viability of probiotics is always sought, promoting responsible production and consumption (SGD-12).

INDEX

1. Introduction

Attention deficit and Hyperactivity disorder (ADHD) is associated with considerable challenges in everyday life, often causing impaired academic outcomes and difficulties within both social and familiar contexts (1).

It is the most common neurodevelopment disorder among children and adolescents (2). This condition affects approximately 6-7% of children in Spain, and the number of cases has been increasing since the pandemic (3). During childhood, boys have two to three times higher prevalence than girls. Interestingly, this difference is not observed in adulthood (4).

The diagnostic criteria for ADHD are defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). There are three possible presentations that may change across the lifespan. These presentations are predominantly inattentive, predominantly hyperactive/impulsive and combined presentation (5).

ADHD is a highly intricate multifactorial disorder which is highly heritable. However, its aetiology is influenced not only by genetics, but also epigenetic and prenatal factors among others. Its physiopathology has been described to be due a deficit of neurotransmitters, mostly dopamine and norepinephrine, although other neurotransmitters are also involved. The lack of neurotransmitters in the synaptic space causes the characteristic symptomatology of this condition. Nevertheless, knowledge gaps are still abundant when explaining the etiopathology (6).

Due to its early appearance, pharmacological treatment is often prescribed at very young ages. However, families are oftentimes reluctant to medicate their children in such an preliminary stage. Moreover, long-term effectiveness of medication-based treatments have been questioned as they fail to meet specific needs of individuals with ADHD (1). Therefore, developing new effective clinical approaches for people with this disorder remains a central aim for researchers within the field.

In the last decade, research into ADHD has increased, leading to new insights and discoveries. This fact arises new treatment possibilities which includes gut-brain axis modulation. In this context, recent studies point that *Bifidobacterium longum* could have a relevant role in ADHD prevention.

Hypothesis: Could a supplementation with *Bifidobacterium longum* subesp *infantis* in formula milk prevent ADHD development?

Objectives:

- To analyse the current understanding of ADHD aetiology.
- To explore the role of the gut-brain-microbiota axis in the development of ADHD.
- To assess the influence of breastfeeding on microbiota composition, immune system maturation and ADHD onset.
- To evaluate the potential of *Bifidobacterium infantis* as a protective factor against ADHD through its immunomodulatory effects.

2. Methods and materials

This thesis is based on extensive bibliographic research from academic databases obtained, such as Pubmed, SpringerLink, Scopus, ScienceDirect and Nature. The literature search was conducted in thematic blocks, applying specific keywords according to the section. Examples of search terms included: "*microbiota-gut-brain axis AND ADHD", "breast feeding AND ADHD*", *"ADHD AND inflammation*" or "*Bifidobacterium infantis AND inflammation".*

To ensure conclusions were drawn from the most current evidence, mostly literature published within the last 10 years was used, prioritizing those studies from the last 5 years. Notably, in the recent years there has been a change in the understanding of pathogenesis of the disorder, which highlight the importance of using updated data. However, a limitation of relying on such recent literature is that several studies are not yet conclusive, and much research is still required in the field.

In addition to literature analysis, expert consultation was carried out in order to enrich the study with multidisciplinary perspective:

- Interview via Microsoft Teams to Himanshu Kumar, senior researcher at Danone Nutricia (Utrecht) and professor at the University of Turku (Finland). With nearly 50 publications on microbiota and breastfeeding, Professor Kumar also investigated the impact of microbiota on neurodevelopmental disorders.
- Telematic interview to Dr Oriol Miquel, gastroenterologist specialized in inflammatory bowel disease, intestinal microbiota and fecal microbiological markers. Since 2012 enrolled in the PhD program where he researches inflammatory bowel disease in order to detect fecal markers of inflammation.
- A virtual meeting was held with Dr Paula Herrera Gener, psychiatry specialized in child and adolescent psychiatrist and eating disorders at Maudsley Hospital.
- Dr Emilio Fortea, a pediatrician at the Bofill Clinic, shared his professional experience and extensive knowledge, offering valuable insights into treatment and multimodal management of the disorder.
- Brief telephone interview was conducted with Águeda Soto, a sales-representative from Nutribiótica, providing a perspective on available probiotics formulation. She presented different probiotic products containing several *Bifidobacterium* species with immunomodulatory effects.

An attempt was also made to contact relevant experts in the field, however, not all of them answered. Among others, an attempt was made to speak with:

- Andreu Prados, pharmacist and nutritionist-dietician specializing in microbiota and professor of the degree of Human Nutrition and Dietetics of the Blanquerna Faculty of Health Sciences of the University of Ramon Llull.
- Dr. Josefina Castro-Fornieles, psychiatrist and psychologist who has participated in the development of clinical practice guidelines on ADHD in children and adolescents.

Finally, as part of the research, a webinar on "probiotics for growing needs" was attended. Organized by Novonesis, it provided interesting insights into the importance of a healthy microbiota in early life. This webinar was particularly interesting for this research as it introduced a supplement on market containing *Bifidobacterium infantis*.

3. Attention Deficit and Hyperactivity Disorder

3.1. Aetiopathogenesis

3.1.1. Genetic factors and neurotransmitter alterations

ADHD symptoms are believed to arise from the dysfunction of neural circuits associated to neurotransmitters impairment. Different genetic variants lead to brain function abnormality, causing concentration difficulties, impulsivity of hyperactivity. Some of the most studied genes related to ADHD are dopamine transporter (DAT1), dopamine receptors (DRD4, DRD5), serotonin transporter (5HTT) and serotonin receptor (5HT1B) (4).

Genome-wide association studies (GWAS) have identified 27 significant risk loci associated with ADHD. A locus is a fixed position on a chromosome where a specific gene or genetic marker is located. Among them, not only are included neurotransmitters-related genes, but also genes associated to the inflammatory response which contributes to the signs manifestation (7).

Dopaminergic and adrenergic pathways are the primary pathways affected in ADHD, although the serotoninergic and cholinergic pathways are also involved to a lesser extent.

ADHD is a disorder that arises as a result of a combination of environmental and genetic factors. Decades of study into ADHD have uncovered multiple chemical alterations that contribute to the condition, such as dysfunction of the dopaminergic pathway. Many of the genetic alterations observed in this condition are linked to dopamine, for instance it is common to detect dysfunction of the D2 receptor gene, D4 receptor gene or dopamine transporter gene (DAT).
The classic hypothesis of ADHD suggests an overexpression of the presynaptic dopamine transporter (DAT), resulting in a reduced availability of dopamine in the synaptic cleft. The current pharmacological approach is based on this theory, since the first-line treatments increase DA levels by inhibiting its reuptake into the presynaptic neuron. However, it is important to note that this theory originated over 40 years ago, and findings from neuroimaging studies using PET and SPECT have produced inconsistent results. These discrepancies may be attributed to methodological limitations and the inherent complexity of interpreting neuroimaging data (8).

Serotonin also plays a determinant role in ADHD due to its close connection with the dopaminergic system. This neurotransmitter crosses the blood-brain barrier in the form of its precursor, tryptophan, and in the brain gives rise to serotonin. In this disorder, there is a decrease in tryptophan transport across the blood-brain barrier, leading to a decrease in serotonin in the brain. Chronic deficient levels of 5-HT have been associated with worsening impulsivity and hyperactivity in ADHD (9).

3.1.2. Environmental factors

Initially, ADHD was thought to be purely dependent on genetic factors. Nevertheless, despite being highly hereditary, recent investigation has shown that ADHD is an heterogenous and multifactorial disorder in which other non-genetic factors are involved. Environmental exposure has been shown to be relevant and contribute between 20 and 30%. It has been found in twin studies that heritability is estimated to be between 80-70%. This mismatch cannot be explained by random mutations alone, but it is believed that other factors are involved in the development of ADHD. Some researchers are studying gene-environment interactions to show whether epigenetic modulations contribute to the development of ADHD. Differential DNA methylation associated with genes involved in neurodevelopment has so far been observed in peripheral tissue. However, because the samples are not from the Central Nervous System (CNS), it is still difficult to establish a causal relationship (6,10).

Causality between environmental exposure and ADHD is hard to demonstrate cause data is obtained from observational instead of experimental studies. In addition, there is correlation between some genetic and environmental factors which further complicates the establishment of causal relationship. It is known that mothers with ADHD are likely to have a poor diet or not take their medication, which are some of the environmental factors that can affect ADHD symptomatology manifestation (11). To put it differently, environmental factors are, in part, a consequence of ADHD itself. Even though, causality cannot be established, there are some prenatal and perinatal risk factors which are reliably associated to ADHD. For instance, intrauterine exposure to tobacco, maternal stress and obesity during gestation and feeding type are linked to this disorder.
Postnatal factors also play a determinant roll in ADHD expression. For example, artificial food colorings and flavoring increase severity of the symptomatology (4).

Establish causal relationships between environmental factors and ADHD is challenging as it is complex to differentiate whether the factor itself causes the disorder or, on the contrary, this factor is related with the parent's ADHD. The following figure illustrates the associations and causal relationships with ADHD and its symptomatology, to provide a better understanding of the complexity of its aetiology.

It has been observed that mothers with ADHD have a higher tendency to have preterm births, which are associated with both gut dysbiosis and ADHD. A cohort study involving nulliparous women giving birth between 2007 and 2014 evaluated the risk of preterm birth in mothers with ADHD. During this period, 6327 women diagnosed with ADHD gave birth and findings indicated risk 40% higher risk of very preterm birth (<32 weeks), even after adjusting for confounding factors such as alcohol consumption (12). Gut dysbiosis is caused by an imbalance of the microbiota and this is closely related to the inflammatory state, as a dysregulation of the microbiota can increase the immune system response.
On the other hand, parents pass on a genetic load to their offspring which include, not only genes related to neurotransmitters, but also genes implicated in inflammatory processes. Owing to this, people with ADHD have a higher baseline level of inflammation which in turn can cause dysbiosis

(13). Moreover, dysbiosis has been associated in some studies with worsening ADHD symptomatology.

When considering the impact of parents in the development and symptomatology of ADHD, apart from genetic load, environmental factors conditioned by the behavioural patterns of parents with ADHD should be considered.(11) For instance, mothers with ADHD, partly due the characteristic symptomatology of the disorder, have a greater tendency to discontinue breastfeeding earlier and their children are more likely to have nutritional deficits. Bringing breastfeeding to an end has an impact on microbiota maturation, especially if it is stopped before three months of age (14). In addition, nutritional deficits are also partly linked to ADHD pathophysiology, stated otherwise, the condition itself lead to nutritional deficits which are associated with worsening symptomatology. This can be attributed to malabsorption and metabolism dysfunctions characteristic in children with ADHD.

The figure outlines the factors influencing the maturation of the gut microbiota, which has been linked to ADHD via the microbiota-gut-brain axis, although a causal association has not yet been demonstrated. Notably, children with ADHD tend to present dysbiosis and a delayed maturation of the gut microbiota (4). Dysbiosis during early life has also been linked to higher risk of the onset of neurodevelopmental disorders (15).

In summary, the aetiology of the disorder is highly complex and understanding the role of the microbiota presents additional challenges. The figure aims to illustrate not only the intricacies of the development, but also the key role of the microbiota, particularly during early life. Numerous risk factors associated with the condition involve alterations in the gut microbiota. Evidence suggests that, in case of premature births, insufficient breastfeeding periods or high levels of inflammation, the balance of the gut microbiota is affected. This disturbance leads to increased systemic inflammation and, subsequently, to a worsening of symptoms.
Moreover, it has also been reported that children with ADHD are prone to alterations of the gut microbiota, which may in part be associated with the hyperactivity of the immune system inherent to the disorder.

Finally, dysbiosis, arising, for instance, from insufficient breastfeeding, may elevate the body inflammatory state of the body which could lead to increased neuroinflammation, thus triggering ADHD. This is particularly noteworthy given, which supports a bidirectional perspective. Put it differently, dysbiosis may not only result from increased inflammation state commonly observed in children with ADHD but could also act as contributing factor. Dysbiosis in early life involve elevated inflammatory state that could affect infant neurodevelopment, triggering ADHD.

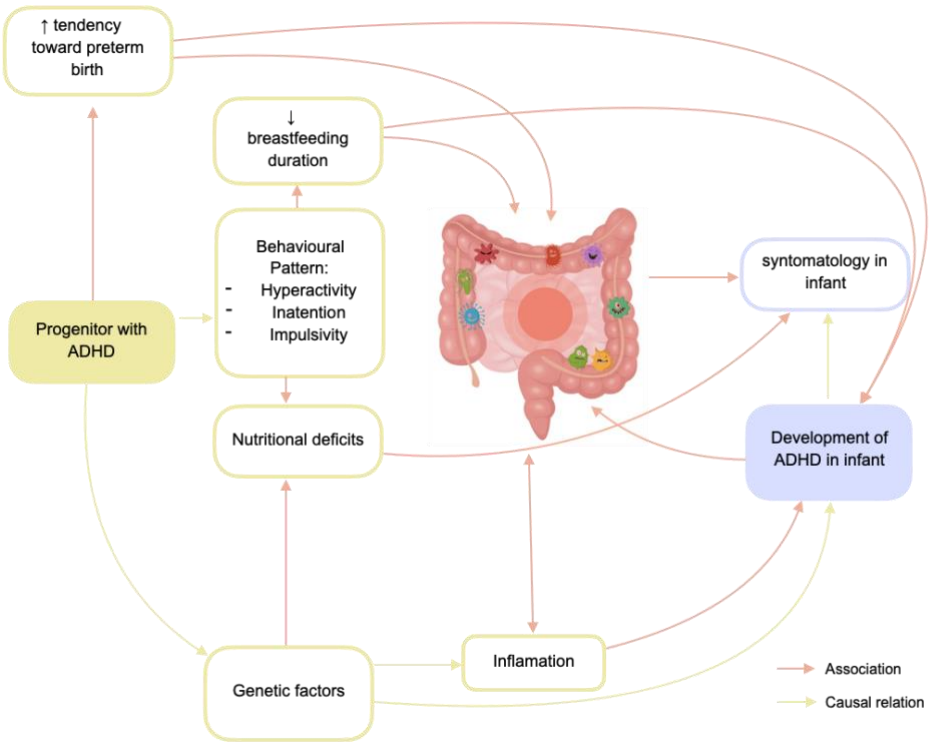

Figure 1:Concept map of ADHD aetiology. Author's own work

3.1.3. Immune system and Neuroinflammation

The classic pathophysiology is related to a deficit of neurotransmitters, especially norepinephrine and dopamine. Additionally, in the past years, more research has been carried out about the association between ADHD and inflammatory processes and how it could affect the etiopathology of the disorder. It has been observed that people diagnosed with inflammatory or autoimmune disorder have 30-50% higher likelihood of developing ADHD which indicates a possible common immune-related underlying mechanism (9).

Substantial evidence that allergic diseases are related to ADHD. It is proposed that the pro-inflammatory cytokines interfere in the maturation of prefrontal cortex, DA mechanism and modifies BBB permeability which affect bioavailability of neurotransmitters. Therefore, neuroinflammation appears as consequence of unbalanced immune response (13).

Accumulation of inflammatory cells in the CNS leads to brain dysfunction. Emerging evidence supports a correlation between the immune response and ADHD, although the involvement of immune cells is yet unclear. A bidirectional Mendelian randomization analysis attempted to reveal the immune cells implication in the pathogenesis of ADHD. The study concluded that, among the various cell types, B cells were the only cells identified as a risk factor for ADHD onset. This cells not only

present antigens to CD4+ T cells, but also synthesise inflammatory cytokines, namely IL-6. This mechanism may explain the increased levels of IL-6 in people with ADHD (16).

Higher levels of pro-inflammatory cytokines IL-1 and IL6, and lower levels of anti-inflammatory cytokines, such as IL-10 (17), have been reported in patients with ADHD. IL-6 has been shown to be involved in learning and memory processes, therefore altered levels may be relevant for neurodevelopment. Moreover, studies in mice have shown that increased IL-6 levels decrease central DA availability and increase dopamine D2 receptor expression (18).

Additionally, Elsadek and colleagues also reported a significant increase of IL-6 levels in serum in ADHD children, however, it was not associated to symptomatology severity. In contrast, significant changes in TNF-alfa were observed (19). Although numerous studies highlight an increase in pro-inflammatory cytokines in children with this condition, the lack of longitudinal studies does not yet allow a causal relationship to be established (18).

Several studies suggest that neuroinflammation may be a consequence of excessive activation of the immune system, leading to chronic oxidative stress. Inflammatory cytokines can cross BBB resulting in inflammation of cerebral tissues. In response to tissular damage, microglia, the principal SNC immune cell, secrete proinflammatory cytokines, leading to increased central inflammatory state. Similar neuroinflammatory processes have been described in other psyquiatric disorder, namely, ASD and depression.

Beside neuroinflammation, oxidative stress should be considered an influential factor in ADHD as an increasing imbalance in oxidative levels has been observed in individuals with this condition. Oxidative stress is the result of disrupted levels of Glutathione (GSH) and increased ROS production. Glutathione is an intracellular antioxidant which neutralize oxidative products. The enzyme glutathione peroxidase is able to neutralise a peroxide by reacting it with GSH. This results in formoglutathione disulphide (GSSG), which must be reduced back to GSH so that GSH peroxidase can continue to catalyse the reaction. New research shows that children with ADHD have lower GSH oxidative imbalance occurs when oxidative products are greater than the antioxidant content. Consequently, lipid oxidation and genetic material damage take place, resulting in a loss of brain function (9).

3.2. Treatment

ADHD is a complex neurodevelopment disorder which mostly affect children. Infants with ADHD face a less optimistic future perspective as they have a higher risk of academic failure. Moreover, hyperactivity and inattention are responsible of more increased rates of car accidents and the transmission of sexually transmitted infections (STIs) (20). In order to achieve better outcomes in the management of ADHD symptomatology, a multimodal treatment approach must be followed.

3.2.1. Pharmacological approach

The treatment of ADHD should be multimodal, that is to say, pharmacological treatment is more effective when combined with psychoeducational and psychological treatment involving parents, teachers and children. Furthermore, before establishing treatment, comorbidities such as autism spectrum disorder (ASD), anxiety or other behavioural disorders should be considered. In general, therapeutic priority is given to the most severe condition. Pharmacological interventions for ADHD are categorized into psychostimulants and non-psychostimulants (21).

The Multimodal Treatment Study of Children with ADHD (MTA) was conducted in response to public unease regarding stimulant treatment. The aim was to determine the effectivity of long-term pharmacological treatments compared to behavioural management strategies and establish intervention guidelines. This study had a great impact in ADHD research as it was the first experimental long-term study which evaluated the different ADHD approaches in a considerably large population (22). While adverse effects from medication are similar among children and adults, infants under 5 of age could be more likely to experience side effects. According to NICE guidelines, pharmacological treatment is recommended in children over 5 years old and methylphenidate is the treatment of election. In the event of poor response, amphetamines would be used. In case of children over 18 years of age, NICE guidelines point that treatment can be initiated with Lisdexamphetamines or methylphenidate (6).

According to Spanish guidelines, pharmacological treatment for children with ADHD should start from the age of six 6 onwards. The initial approach should involve psychostimulant medication, with methylphenidate and lisdexamfetamine being the two approved options in Spain (23). Methylphenidate acts as a DA and NA transporter blocker and is commercialised in different pharmaceutical forms. There is extended-release, immediate-release or osmotically controlled extended-release methylphenidate, the main difference being the duration of effect. This drug would be the first-line treatment drug of choice. In case of insufficient response, the preferred alternative would be lisdexamfetamine. This amphetamine is the only one approved in Spain and is an adrenergic agonist targeting the DA and NA system (24).
Among the non-psychostimulant options available, the recommended order of use is atomoxetine, guanfacine, and clonidine (23). Atomoxetine is a selective NA reuptake inhibitor with a 24-hour duration of effect. Guanfacine and clonidine are alpha-adrenergic agonists, with guanfacine being more selective and generally associated with fewer adverse effects (24).

In addition, several drugs with new therapeutic targets are currently being studied as alternatives to conventional ADHD treatment. One such compound is Dexmedetomidine (DEX), an anesthetic and α-2 adrenergic receptor agonist, pharmacologically related to guanfacine which is already used to treat ADHD. DEX has been studied in spontaneously hypertensive rats, which have ADHD-like behaviour. This drug is an anesthetic of the α-2 adrenergic agonist family, as is guanfacine, used in the treatment of ADHD. Beyond its effects on hyperactivity, the drug appears to influence on the gut microbiota. It can modify it, promoting the growth of bacterial genera with anti-inflammatory effects. DEX administration decreased IL-1, IL-6 and TNF-α levels and increased IL-10 levels. These results

suggest that by modulating the microbiota, it decreases inflammation at both intestinal an CNS levels, leading to improved brain function.

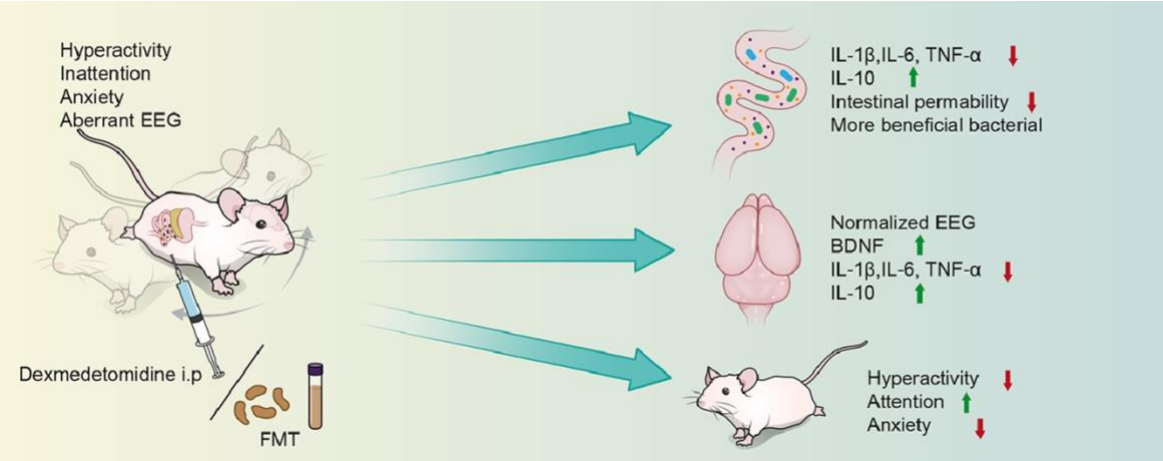

Figure 2: Dexemedetomidine administration in mice: immunomodulation and attenuation of ADHD-like symptoms (25)

To confirm that the benefits of DEX are attributed to modulation of the gut microbiota, fecal microbiota transplant was performed on rats previously treated with antibiotics. A portion of them received the microbiota from DEX-treated rats while the rest received only a saline solution. The rats that received the transplant from DEX-treated had lower levels of pro-inflammatory cytokines. These findings support the conclusion that the gut microbiota is a potential therapeutic target for ADHD, as it has an impact on the CNS through the microbiota-gut-brain axis (25).

3.2.2. Nutritional approach

Role of Diet in ADHD management

Diet also influences on the expression and possible development of ADHD. As discussed in previous sections, the microbiota plays a crucial role in the control of inflammation and ADHD symptoms. The composition of the gut microbiota is closely linked to the type of diet and therefore, a healthy diet will promote a more balanced microbiota. The Western diet is characterised by a high intake of saturated fats, simple sugars and low fibre. This type of diet has been shown to decrease the population of *Faecalibacterium*, which can modulate the immune system. This condition has also been observed in children with ADHD and it has been proposed that this decrease may be implicated in the development of the disorder. This can be attributed to because an increased inflammation state and overproduction of pro-inflammatory cytokines, which are capable of crossing the BBB and affect neurodevelopment (26,27).

Although there are numerous nutritional recommendations that claim to be beneficial for ADHD, NICE guidelines only support a varied and balanced diet, such as the Mediterranean diet. To date, there is not enough high-quality evidence to recommend omega-3 and omega-6 supplementation. That said, the “few foods” diet has been studied in children with ADHD, however, it should not be recommended

either. While it appears to have a positive impact, present studies are not conclusive as they have multiple limitations, including the small number of participants. This diet is very restrictive and the long-term consequences of following this diet at growing ages have not yet been evaluated. Furthermore, initial elimination of artificial colours should not be advised, instead it should be detected whether any food or drink increases hyperactivity (28).

Nutrients supplementation

It has been proved that dietary intervention has an impact in ADHD management.

ADHD patients are more likely to be deficient in certain micronutrients due absorption and metabolization alterations. As mentioned in a previous section, these deficits result in symptomatology worsening. Zinc and Iron are specially associated to ADHD physiopathology as are involved in the production of noradrenaline and dopamine, crucial neurotransmitters which are fundamentally altered in ADHD. As consequence, lower levels of this compounds could lead to brain dysfunction. Furthermore, vitamin D levels are as well typically decreased in ADHD patients.

Therefore, whenever a new case of ADHD is diagnosed, zinc, iron and vitamin D levels should be assessed in a blood test. In case of low levels, supplementation should be initiated until restoring normality values. It is worth considering that, due the tendency to be deficient in certain micronutrients, the minimum values for these micronutrients should be higher than for general population. Zinc, iron and vitamin D are the most investigated supplementation in ADHD (29).

Iron is crucial cofactor for the immune response, cellular respiration and oxygen transport. In addition, iron plays an important role in DNA synthesis and Iron is cofactors implied in the production of dopamine and noradrenaline, therefore abnormal levels could impact in ADHD signs. It is involved in the proper functioning of enzymes implied in the synthesis and degradation of dopamine. Recent research has shown that iron levels during the first 2 years of age are crucial cause a deficit of iron is linked to CNS development and behavioural and psychomotor problems. When considering the efficacy of micronutrients supplementation in ADHD, subtype of the disorder must be taken into account. For instance, Soto-Insuga and colleagues observed higher efficacy of Iron supplementation in children with inattention subtype of ADHD, although the little number of participants conditionate the validity of results (30).

Iron deficiency based on ferritin levels is highly variable because reference values change depending on the individual's inflammatory state. The World Health Organization (WHO) defined in 2020 iron depletion as ferrition levels below 12 µg/L of ferritin /ml in children under five years old are considered iron depletion. This value is increased to 15µg/L for children over 5 years of age and in presence of inflammation, the threshold used is 30µg/L (31). It is the amount of ferritin found in the cerebrospinal fluid which really indicates the need to initiate supplementation (30). Iron deficiency is more common in children with ADHD, and it is associated with impaired functioning of the dopaminergic system in the CNS. In addition, the literature describes a worsening of ADHD symptomatology in children with

iron deficiency. Given the predisposition to iron depletion, the minimum recommended ferritin concentration in children with ADHD should be higher than for those without the disorder. For instance, in the study conducted by Soto-Insuga and colleagues, hypoferremia in ADHD is described as ferritin values below 30 ng/ml (30).

On the other hand, Zinc is an essential trace element. It is related to the metabolism of melatonin, neurotransmitters and prostaglandins. Even though the little amount of zinc required for the well-function of the body, it play a determinant role. It is implied in growth and development, immune system and DNA synthesis. Additionally, is crucial in the metabolism of melatonin which has a determinant role in dopamine regulation. As a consequence, this micronutrient is considered a dopamine reuptake inhibitor. Moreover, zinc has been proposed to be involved in the activation of dietary pyridoxine which assists the conversion of tryptophan to serotonin (32).

Studies have led to controversial results when evaluating the effects of zinc, iron and vitamin D supplementation. It has been proposed that subjects with ADHD have metabolism and absorption alterations which lead to zinc deficit. Arnold et al assessed urinary zinc excretion and found that almost all the administered dose was eliminated on the same day. A fast-release formulation was given in the morning, therefore beneficial effects were observed for only a few hours, until the afternoon, by which the time it had been eliminated by the urine (33). This is thought to be the reason why, when evaluating ADHD signs, only teachers stand out and improvement, while parents reported no effects. In addition, methylphenidate is believed to contribute to lowering zinc levels (34,35). Therefore, zinc supplementation has shown a positive impact in ADHD owing to its implication in the dopamine reuptake, nevertheless, the effect is limited due the absorption alterations commonly found in children with this disorder.

The systematic review by Granero et al concludes that an association has been established between iron and zinc supplementation and the management of ADHD symptomatology. However, the specific role of each nutrient in managing the disorder has not yet been determined and more research is required. It is worth highlighting that more consistent evidence has been found regarding the effectiveness of zinc supplementation in children with ADHD (32).

As to nutrient recommendations, the clinical practice guidelines published by the Ministry of Health indicate that there is insufficient evidence to support zinc supplementation. However, it has been observed that iron supplementation in children with low ferritin levels may be beneficial (23).

4. Impact of microbiota in ADHD

4.1. Gut dysbiosis in children with ADHD

Before discussing the systemic impact of the microbiota, it is first essential to provide an overview of its functions and composition. The gut microbiota consists of approximately 10^{13} microorganisms of different species which perform certain functions in the body and their abundance in the gut exceeds

the number of human cells. This large population inhabiting the gut confers a wide genetic variety and complexity that contributes to the homeostasis of the body. The balance of the microbiome is key to promote an individual's state of health. The intestinal microbiota is comprised of more than 90% of the following phyla: Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, the predominant bacteria being Firmicutes and Bacteroidetes. The remaining percentage corresponds to Fusobacteria and Verrucomicrobia. The abundance of certain species is conditioned by the degree of stress, hormonal changes and type of feeding, among others (36).

The gut microbiota plays a determinant role in the digestion and absorption of nutrients, among other functions. It contributes to the proper functioning of intestinal motility, regulate pH by preventing the growth of pathogens and produce numerous metabolites that have an impact beyond the digestive system. These metabolites include vitamins, short-chain fatty acids (SCFA) and neurotransmitters or their precursors.
SCFA contain less than six carbon atoms, and they are produced by anaerobic bacteria through the fermentation of dietetic fibre and amino acids. The most common SCFA are formic acid, acetic acid, propionic acid and butyric acid. They are used as energy source for the cells of the colonic epithelium, and, moreover, SCFA act as neurotransmitters and immunomodulators. Lastly, SCFA are involved in mitochondrial functions and cell proliferation and differentiation through the interaction with GPR receptors and inhibition of histone deacetylase. These fatty acids can modulate the neural proliferation and, butyric acid is specifically involved in colonocytes proliferation (37).

Through the production of these metabolites the microbiota communicates with the rest of the body, via stimulation of the vagus nerve, enteric nervous system (ENS), immune system and hormonal pathway. It can activate the immune system through pro-inflammatory products and while also may have an immunomodulatory effect. Finally, the intestinal microbiota's capabilities include neutralisation of toxins (38).

First years of life are crucial as the entire body undergoes continuous maturation. There is simultaneous development of central nervous system and the gut microbiota, and each can influence the development of the other. In addition, studies show that dysbiosis at an early age can cause physiological and psychological disturbances.

In the evolving field of microbiota research in children with ADHD, recent studies have observed a clear tendency towards dysbiosis. It should be considered that the microbiota varies according to multiple environmental factors such as diet, antibiotic treatment, exercise and stress, among others. For this reason, it is challenging to determine in general terms which species are altered in cases of ADHD.

The analysis of gut microbiota is very complex, as studies often evaluate different species and genera to determine dysbiosis, therefore, drawing consistent conclusions is a challenge. To address this, a comparative analysis of the current literature on gut microbiota in children with ADHD was conducted. Only studies published within the last 10 years were included and contrasted with the findings of

systematic reviews published from 2022 onwards. This approach ensured that the results were evaluated against the most recent and relevant data available (9,39–41).
Moreover, the findings were set against the conclusions drawn from the presentation on psychobiotics and mental disorders by Dr. Martin Carrasco during the 5th Danone Workshop (42).
It was concluded that at the phylum level, Bacteroidetes, Fusobacteria and Proteobacteria are increased, while Firmicutes are decreased. Actinobacteria is one of the most problematic and demanding phyla to interpret, as the available studies report contradictory findings. Aarts et al. is the only study included in the table that identifies a significant increase in actinobacteria, attributing this result to the high sensitivity of the analytical method used. Nevertheless, this study does not report whether participants were using probiotics, therefore observed increase could be attributed to supplementation. Notably, despite the apparent increase in this phylum during adolescence, significant decrease in actinobacteria in children with ADHD has been reported. This pattern has been linked to a late maturation of the microbiota. In this study a decrease of actinobacteria associated with a late maturation of the microbiota is observed (43).

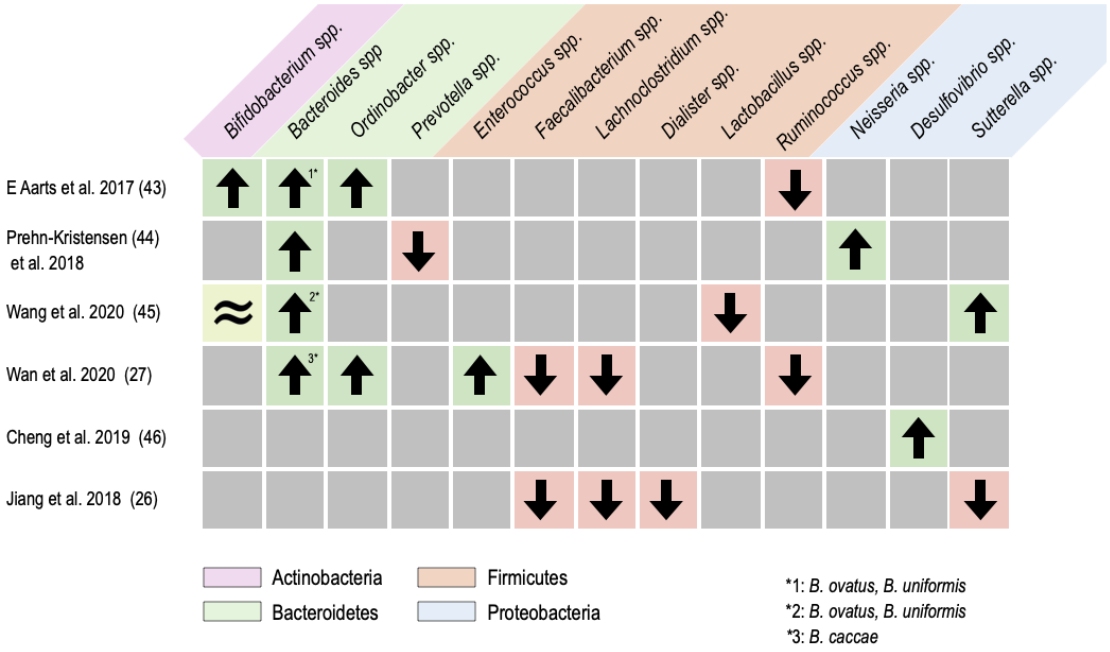

Figure 3: Comparative Analysis of Gut Microbiota at the Genus Level, Grouped by Phylum, in Children with ADHD. Author's own work

Four of the six studies analyse the abundance of different genera within the phylum Bacteroidetes (27,43–45). Among these, two reported a significant increase of more than one genus belonging to this phylum (27,43). Prehn-kristensen et al. indicate that higher levels of Bacteroidetes correlate with increased impulsivity and hyperactivity (44). Similarly, Liang-Jen Wang et al. also describe the influence of increased Bacteroidetes genus on brain development and ADHD pathophysiology, although their findings did not reach statical significance (45). Additionally, an increase in *Ordinobacter* spp., another genus within the Bacteroidetes phylum, has been associated with neurodevelopmental disorders and reduced activity in the brain's reward system (27).

Five studies examined the abundance of various species within the phylum Firmicutes (26,27,43–45). Although a clear decrease of the phylum is evidenced when studying the genera, results become inconsitent when assessing different orders within the phylum. Stated differently, as broader taxonomic classifications are evaluated, the observed differences tend to diminish. Findings across these studies are in part inconsistent, as some genera have increased and others have decreased, making the interpretation challenging. For instance, Cheng et al. 2019 reports higher abundance of Clostridiales (46), whereas E Aarts et al indicates a decrease (43). It should be noted that the study conducted by Cheng et al. is based on gut microbiota data published in the genome-wide association study (GWAS), which aimed to detect associations between gut microbiota and ADHD. This genetic approach helps minimising the confounding effects of environmental and dietary factors. However, the study data are limited as only a small portion of the microbiota could be assessed (46).

This figure illustrates the results obtained from the same previous studies but now assessing the orders. It should be noted that in numerous studies the difference between genera is evaluated but not between orders. In addition, when the orders are compared the data obtained are not so conclusive.

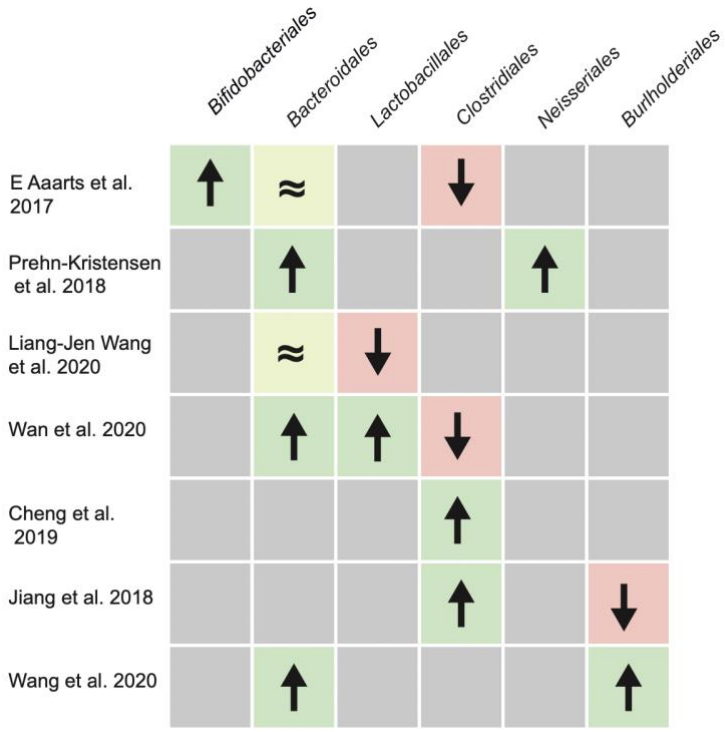

Figure 4: Abundance order changes in TDAH infants. Author's own work

Despite an increase in certain species such as *Enterococcus* spp., there seems to be a trend towards a decrease in Firmicutes. As previously discussed, the abundance of *Faecalibacterium* is consistently found to be reduced in children with ADHD. This alteration has been supported in both studies that analysed the genus. A decrease of *Faecalibacterium* has been linked impaired immune regulation, leading to an increase in proinflammatory cytokines production. These cytokines can cross the BBB and potentially impact on neurodevelopment (26,27).

Within the Proteobacteria phylum, *Neisseria* is the main genus studied when assessing gut dysbiosis in people with ADHD. Two studies evaluate its abundance and only one reported significant result. Prehn-kristensen et al. suggest that the increase in *Neisseria* may be linked to altered sialic acid metabolism in children with ADHD. *Neisseria* could take advantage of these metabolic alterations to

evade the host's immune defends, which may explain its overrepresentation in the gut microbiota (44).

4.2. The Gut-Brain axis in infant neurodevelopment

To understand the impact of the gut microbiome on infant's neurodevelopment, a brief description of gut-brain axis is convenient.

1. Neurological pathway
2. Metabolic pathway
3. Immune pathway
4. Hormonal pathway

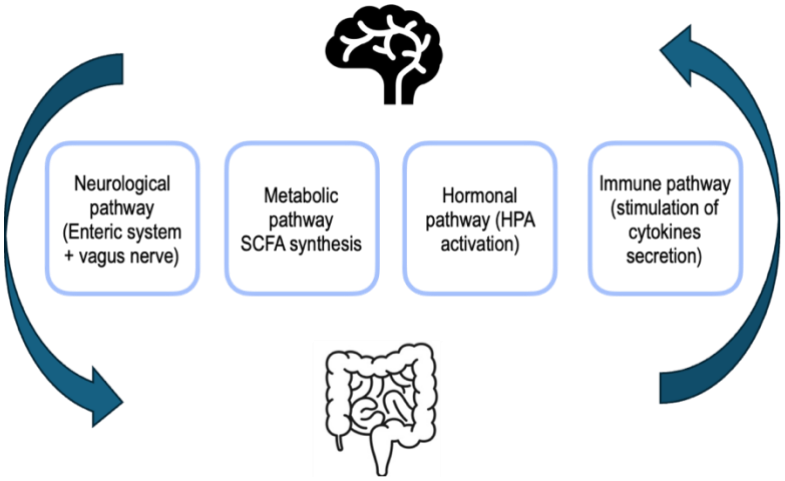

Figure 5: Illustration of gut-brain axis. Author's own work

Neurological pathway

Initially, it was believed that information travelled mainly from the brain to the intestine, for instance, stomach pain as consequence of anxiety. Nevertheless, current literature has shown that 90% of the impulses travel from the gut to the brain, rather than the other way around (38). Communication between the microbiota and the CNS is mainly established through the vagus nerve, although the ENS is also involved.

Furthermore, the ENS plays a determinant role in the gut-brain axis. This network of neurons is often referred as a second brain, as it can process the information received via afferent neurons and generating a response independently. Among its functions, the ENS provides local reflex responses and contributes to regulate peristalsis. It also communicates with the CNS either through the vagus nerve or via the secretion of immune signaling molecules (47).

Metabolic pathway

Gut microbiota synthesises different neurotransmitters (NTT), for instance, dopamine, glutamate, GABA and histamine. In addition, they produce neuroactive components such as nitric oxide, short chain fatty acids (SCFAs) and tryptophan. These metabolites can modulate immune system, and gastrointestinal motility, apart from activating vagus nerve, enteric nervous system and enterochromaffin cells. Compounds synthesised by the microbiota are detected by afferent neurons of the enteric nervous system (ENS). The ENS is connected to the vagus nerve and, simultaneously, it can autonomously respond to signals received from the microbiota and can stimulate enteroendocrine cells, thereby modulating the release of serotonin and other hormones. Furthermore, enteroendocrine cells can also stimulate the vagus nerve (36).

The figure below illustrates how the microbiota can synthesise precursors of key neurotransmitters in ADHD, such as serotonin, dopamine and noradrenaline, thereby influencing their production. While

gut neurotransmitters are unable to cross the BBB, their precursors are capable of doing so (43). Consequently, certain bacterial genus including *Bacillus* spp., *Escherichia* spp. and *Streptococcus* spp. may have a positive impact on the production of these neurotransmitters at the CNS level, potentially affecting the symptomatology of the disorder (48). It is worth noting that *Streptococcus* spp. and *Bacillus* spp. belong to Firmicutes phylum, which is commonly reduced in individuals with ADHD.

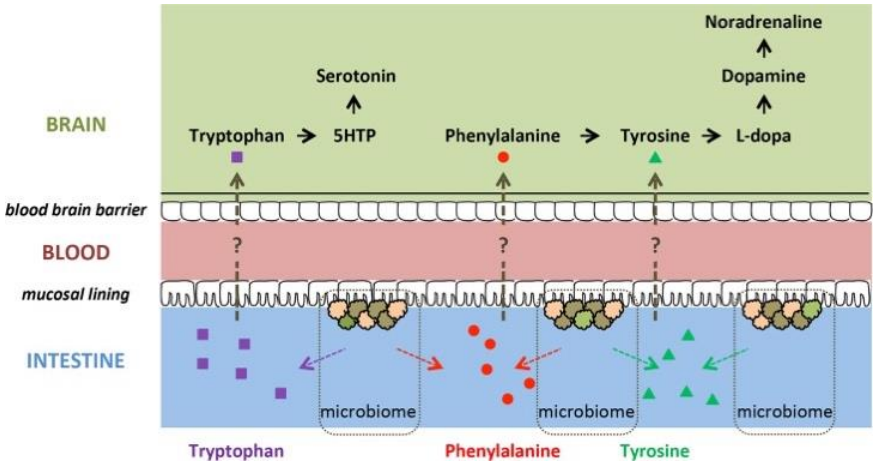

Figure 6: Influence on brain functioning of microbiota produced monoamines (43)

There is increasing evidence that production of NTTs and metabolites by the microbiota can modulate neurodevelopment. Several studies suggest that *L. rhamnosus* may improve the integrity of the intestinal barrier and regulate the secretion of neurotransmitters via the vagus nerve, resulting in a reduction of ADHD symptoms. Furthermore, a negative correlation has been identified between a metabolite called 4EP(S) and neurodevelopment. This compound is of particular interest because it is produced by *Bacteroides ovatus*, a species increased in children with ADHD (49).

Hormonal pathway

Hypothalamic-Pituitary-Adrenal (HPA) axis plays a central role in stress regulation and is involved in the gut-brain microbiota axis. Cortisol release is regulated by the CNS through the HPA axis and influences the microbiota-gut-brain axis via its action on intestinal cells. Epithelial, immune and enteroendocrine cells all have cortisol receptors. This hormone stimulates the release of proinflammatory cytokines such as IL-6 and IL-8 which activate the inflammatory response and increase BBB permeability, thereby inducing neuroinflammation. In chronic stress, there is sustained cortisol secretion which may indirectly affect the microbiota through changes in nutrient bioavailability and absorption, leading to dysbiosis. In addition, microbiota can also activate cortisol release through the vagus nerve and ENS (47,50).

Immune pathway

This pathway is particularly interesting for ADHD as chronic inflammation is increasingly associated with the development of the disorder. Closely related to this, numerous studies link dysbiosis with neurological disorders. Gut microbiota alterations have been observed in multiple neuropsychiatric disorders, including ASD and ADHD. Although research on dysbiosis in children with ADHD is in its early stages, there seems to be a clear association between the inflammatory state linked to dysbiosis and neurodevelopment in these children. The gut microbiota has an important immunomodulatory effect, given that a large portion of the immune cells are located in the gastrointestinal tract. An imbalance of the intestinal microbiota affects the permeability of the intestinal barrier, promoting systemic inflammation. As a result, the most recent studies consider that gut microbiota mat be involved in the etiopathogenesis of the disorder.

The intestinal microbiota, in addition to interacting with the HPA axis, modulates the inflammatory response through different pathways. It can induce the maturation of regulatory T cells and inhibits the synthesis of inflammatory T helper cells. Moreover, the microbiota regulates the production of pro- and anti-inflammatory cytokines through SCFA. These metabolic products, besides being immunomodulatory, they can regulate the tight junctions and reduce gut permeability (9,49).

Intestinal epithelial cells have immune receptors, including Toll-like receptors (TLRs), which detect changes in the microbiota and contributes to the maintenance of immune balance. Immune cytokines are essential for internal regulation, however, an imbalance in the microbiota can lead to excessive production of proinflammatory cytokines. These cytokines can stimulate the vagus nerve, activate the HPA axis or pass into the bloodstream. In addition, they reduce the expression of tight junction proteins in the intestinal barrier, promoting increased systemic inflammation. As a result, there is a decrease in the expression of these proteins in the brain, thereby contributing to neuroinflammation and neurodevelopment problems. In fact, learning disabilities have been linked to chronic intestinal inflammation (47).

One genus notably reduced in dysbiosis is *Faecalibacterium*, which also is in lower abundance in children with ADHD. This genus stimulates anti-inflammatory processes and has anti-inflammatory effects.

Ensuring a correct formation of the microbiota during early life is crucial, as there is a simultaneous development of the CNS and there is a bidirectional influence between the gut and the brain. Growing evidence shows that the traditional models of ADHD pathogenesis are incomplete, and that inflammation plays a determinant role in the disorder. Dysbiosis reduces the integrity of the intestinal barrier resulting in chronic inflammation and overproduction of proinflammatory cytokines. This leads to BBB disruption and ultimately contributes to neuroinflammation, potentially influencing ADHD development (9).

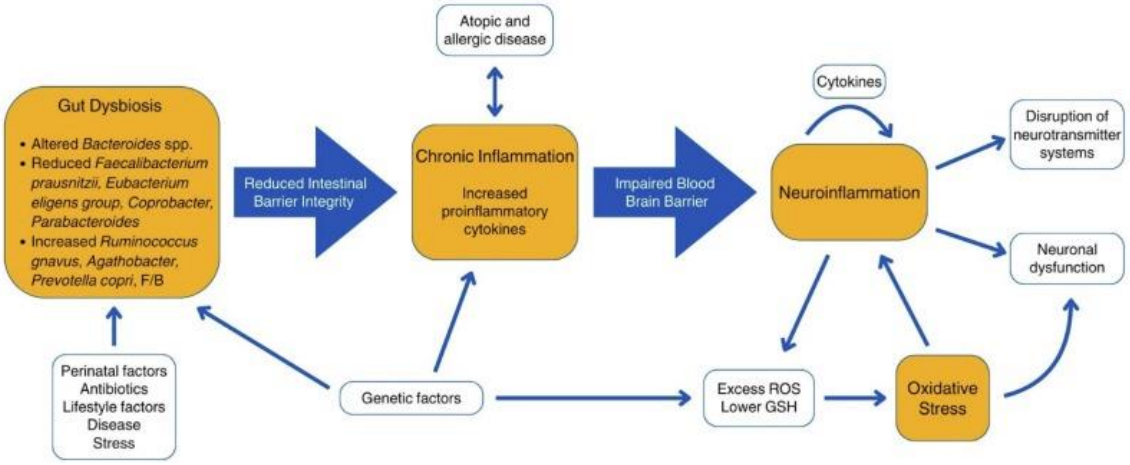

Figure 7: Neuroinflammation as a result of dysbiosis (9)

Moreover, the microbiota influences neurodevelopment through its effect on microglia, the brain's resident phagocytic cells. Microglia has a determinant role in neurogenesis and, therefore, essential for proper neurodevelopment. The microbiota and some of their metabolites such as SCFA, are involved in the maturation and aging of microglia (49).

5. Breast-feeding and ADHD

According to the World Health Organization (WHO) and UNICEF, exclusive breast milk is the optimal food source for newborns during the first six month of age. Thereafter, breastfeeding (BF) should be continued while gradually introducing solid food to diet for up to 2 years. As reported by WHO, during the years 2015 and 2020 only 44% of worldwide newborns between 0 and 6 months were given breastmilk as sole nutrient source (51).

LAyDI, a Cohort study conducted in Spain by AEPap and group PAPenRED, evaluated the prevalence of Breastfeeding in 1,946 newborns for 2 years. Despite the fact that initially 90% of the mothers intent to breast-feed their children, after 15 days the percentage of exclusive BF drops to 66,4%, and after 6 months, the prevalence of breast-feeding is 35,2%. After 6 month the prevalence of BF (exclusive or combined with formula milk) is 54,3% (52).

The increasing formula milk industry has a great impact in the prevalence of BF. A study conducted in US in 2008 revealed that 67% of the mothers who received formula milk samples, breastfed during shorter period (53).

Breastfeeding has a major impact in children health as is associated to less autoimmune disease incidence, allergic diseases or atopic dermatitis. Moreover, it influences early cortical development and is linked to child obesity and diabetes mellitus (54).

A meta-analysis evaluated the incidence of ADHD in children who stopped BF under or over 3 months. It concluded that those children who stopped breastfeeding before 3 months had higher risk of attention deficit and hyperactivity disorder. Moreover, there is a positive association between ADHD and non-breastfeeding. It was proposed that the short length of breastfeeding could also be linked to the hyperactivity of the children (14).

Similarly, a study conducted by H Kim et al. evaluated the association between breastfeeding and the prevalence of various high incidence childhood diseases. It was observed that breastfeeding for 4 to 6 month was a protector factor to ADHD as, after two years, it significantly reduced risk of being diagnosed (54).

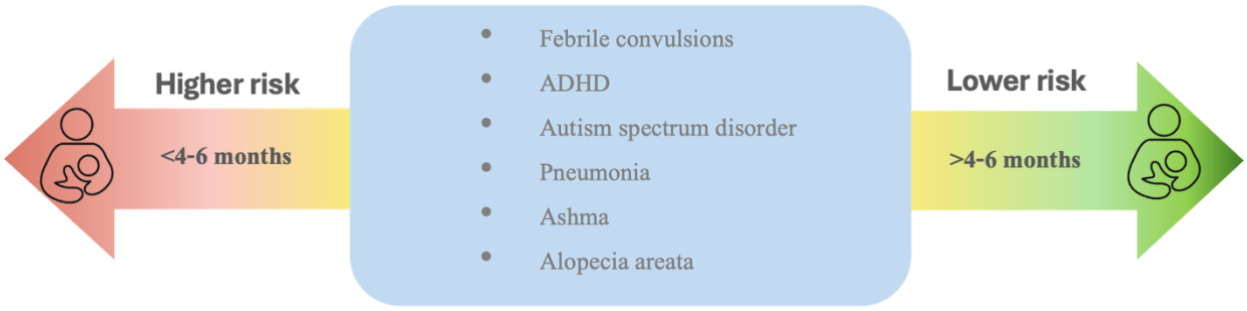

Figure 8:Effects of breastfeeding on childhood diseases, based on Kim et al. 2021 (54)

On top of that, Shamberger et al. observed significant inverse relationship between breast feeding and ADHD incidence. Results showed that a duration lower than 3 months of exclusive breastfeeding is associated with higher hyperactive symptoms. This protective effect was attributed to the DHA and EPA content of breast milk, although it concludes that there is no direct evidence to support this link. It is worth mentioning that the study is dated and DHA and EPA levels in infant formulas are now content standardized to over meet nutritional requirements. However, the rising prevalence of ADHD suggests that other factors may contribute to the development of ADHD, and the protective effect of breastfeeding could be related to other of its components (55).

Emerging evidence reveal that optimal maturation of the microbiota impacts on infant's neurodevelopment, as both processes are simultaneous and interconnected. Accordingly, deep understanding of infant microbiota and symbiosis is essential to ensure its proper development, especially when breastfeeding is not possible (9).

The GI tract is colonized first by facultative anaerobes, such *as Staphylococcus, Streptococcus, Enterobacteriaceae* and *Lactobacillus*. This first colonization enables other obligate anaerobes to grow, namely *Bifidobacterium*, *Clostridium, Bacteroides* (56).
BF is associated with higher abundance of *Lactobacillus* and lower abundance of *Proteobacteria*, *Clostridium* and *Bacteroides*. Pärtty et al found that children with lower abundance of *Bifidobacterium* were more likely to develop ADHD (57).

BF is determinant for the successful development of the microbiota due to the presence human milk oligosaccharides (HMO), which act as prebiotics by serving as substrates for specific beneficial bacterial species. This selective stimulation of microbial growth promotes an appropriate maturation of the gut microbiota.

HMO are considerably abundant in breast milk, representing approximately 20% of its total carbohydrates content and ranking as the third most prevalent component after lactose and lipids. Their concentration is especially elevated in colostrum, containing from 9 to 22 g/L, and gradually decreases as lactation progresses. For the moment, 200 different types have been identified and are divided into 3 groups: fucosylated HMOs, which includes 2'-FL and 3'-FL; sialylated HMOs, which includes 3'SL and 6'SL; and non-fucosylated HMOs, which includes lacto-N-tetraose (LNT) and lacto-N-neotetraose (LNnT) (58).

There are three subspecies of *Bifidobacterium longum* which have been identified as human gut colonising: BL*longum,* BLi*nfantis* and BL*iuvenis.* *B. longum* subsp *infantis* (BLinfantis) is more specific for HMO and N-glycans metabolism, whereas *B. longum* subsp *longum* (BL longum) is specialized into more mature diet components breakdown, such as plant-derived carbohydrates degradation.

N. pucci and colleagues analysed samples of mother-infant pair from the Amsterdam Infant Microbiome Study in order to determine *Bifidobacterium* abundance during the first months of life. The study observed higher abundance of BLlongum the first month after birth, and, after 6 months, higher abundance of BLinfantis. This fact was attributed to the higher amount of HMO and N-glycans from breast milk after 6 months. It was expected to observe larger amount of BLlongum after introducing solid foods, however, it did not occur. The cause might be due insufficient intake of solid food which does not enable BLlongum to outcompete BLinfantis (59).
The following figure illustrates the transition of the abundance of different *B. longum* superspecies during the first and sixth months of life. It is clearly observed that, although *B. infantis* is initially a minority, its relative abundance increases exponentially within 6 months.

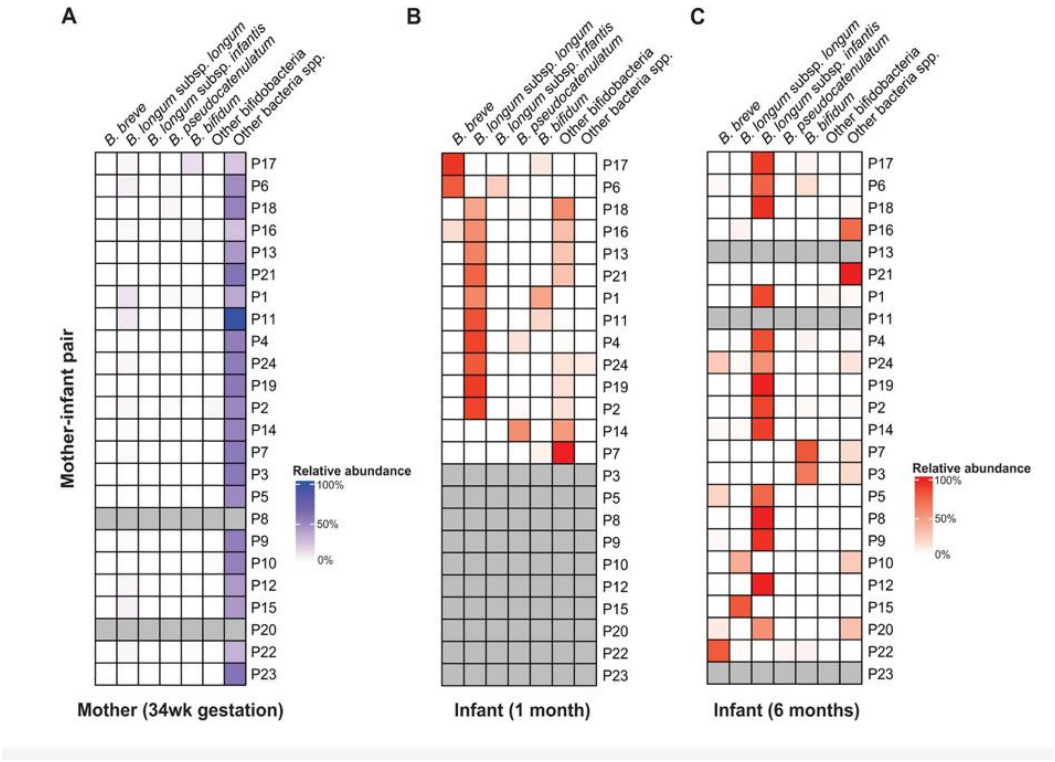

Figure 9: Relative Abundance of Bifidobacterium Species in Mother–Infant Pairs Across Early Developmental Stages (59)

Breast-milk bacteria stimulates antigenic response and, therefore, it promotes T cell differentiation. Not only breast-milk bacteria promote a correct immune system maturation, but in addition enable digestive processes through the Human Milk Oligosaccharides (HMO) digestion. As a result of HMO metabolization, bacteria produce SCFA which are beneficial for immune system and participates in the gut-brain axis (60).

Bifidobacterium longum subsp *infantis* is considered the most efficient HMO cataboliser. As result of HMO utilisation, luminal pH decreases and there is synthesis of lactate, formate and acetate, which can be metabolised into SCFA (59).

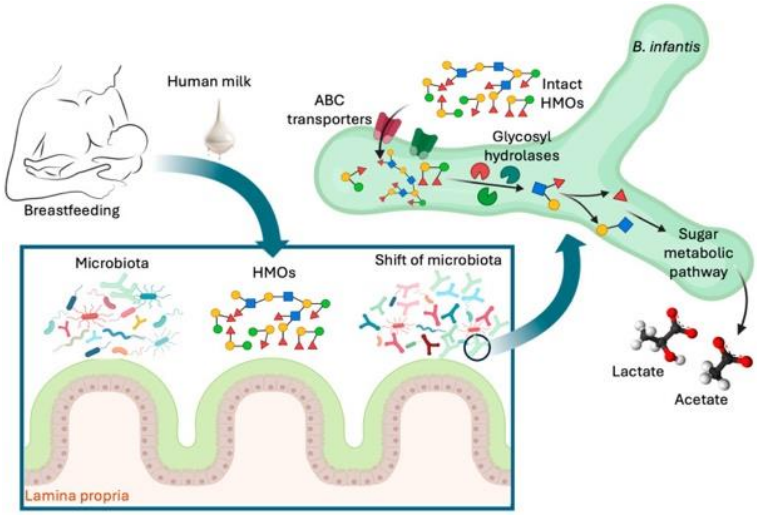

Figure 10: HMO utilization by B. infantis (62)

6. Potential of *Bifidobacterium infantis* supplementation in ADHD prevention

The formation of the gut microbiota has an impact on physical, cognitive and immunological development. For this reason, microbiota colonisation during infancy, and especially the first 3 months of life, is essential. Dysbiosis in infants can result from factors such as mode of feeding, birth method, maternal microbiota and exposure to antibiotics, among others. As a result, the child may have a higher risk of suffering from pathologies associated to an altered immune system, as atypical skin and type I diabetes (15,61). Infant microbiota is strongly marked by an abundance of *Bifidobacterium*, however, it is determined by maternal vertical and horizontal transmission among children. Nevertheless, regardless of the mode of feeding, the abundance of *B. infantis* is reduced in the infant gut. One possible explanation is the absence of this bacterium in the mother's gastrointestinal tract, potentially due to prior antibiotic treatment. If *Bifidobacterium* is decreased, defective vertical transmission will take place. Furthermore, the low rate of breastfeeding in industrialised regions further hinders the establishment of *B. infantis* in the infant gut. Although the composition of formula milk increasingly resembles that of breast milk, notable differences remain that may contribute to dysbiosis (62).

An insufficient population of *Bifidobacterium* during infancy has been connected to an increased risk of immune system disorders, increased inflammatory status and autoimmunity. Supplementation with *B. infantis* has been shown to reduce faecal calprotectin, a recognized marker of intestinal inflammation. A lack of Bifidobacteriaceae has been linked to higher levels of neutrophils, basophils, memory CD8+ T cells, indicating the involvement of both the innate and adaptive immune system (61).

Probiotic supplementation aims to ensure microbial balance, thereby promoting the optimal maturation of the immune system. Due to the major impact of the inflammatory state on the aetiopathology of ADHD, the correct maturation of the immune system could reduce the risk of developing the disorder.

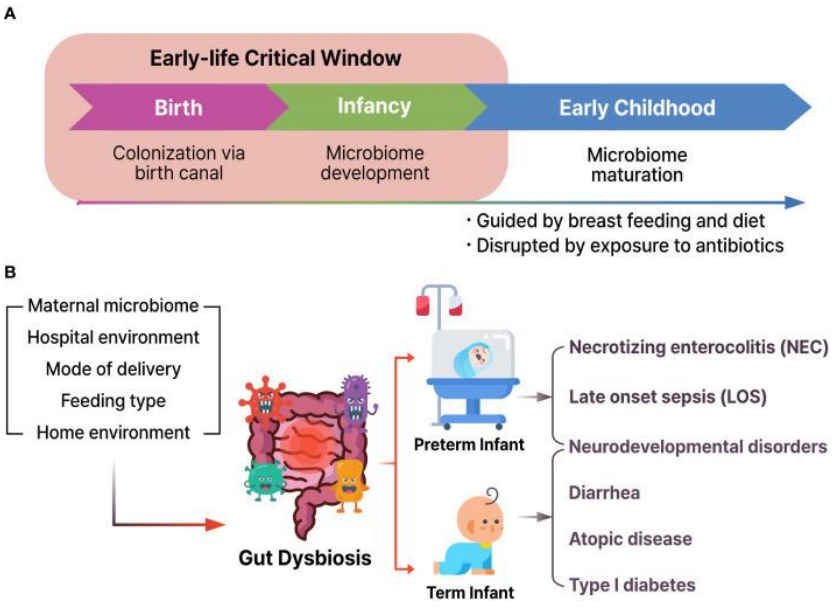

Figure 11: Early-life gut microbial development (15)

B. infantis is the primary catabolizer of HMO and, as a result, produces a large number of metabolites, among which are lactate, acetate and indole-3-lacic acid (ILA). ILA participates in tryptophan catabolism and has immunomodulatory effects. This metabolite (ILA) can bind to the aryl hydrocarbon

receptor (AhR), an important receptor involved in controlling intestinal homeostasis and immune responses and modulate the LPS response (63). Moreover, ILA modulates the response of Th2 and Th17 cells, minimizing systemic inflammation. Reduced utilization of HMO has been linked to increased levels of proinflammatory cytokines (61). ILA is hypothesized to upregulate galecti-1 in T cells and, consequently, exerts an anti-inflammatory effect by modulating Treg cells. Furthermore, SCFA generated by *B. infantis* contribute to the development of the immune system by stimulating of the secretion of anti-inflammatory cytokines. *B. infantis* is therefore crucial for maintaining the integrity of the intestinal barrier and promoting the immune system development (64). Consequently, a lack of *B. infantis* is associated with immunity system deregulation and presence of an increased number of intestinal pathogens (56).

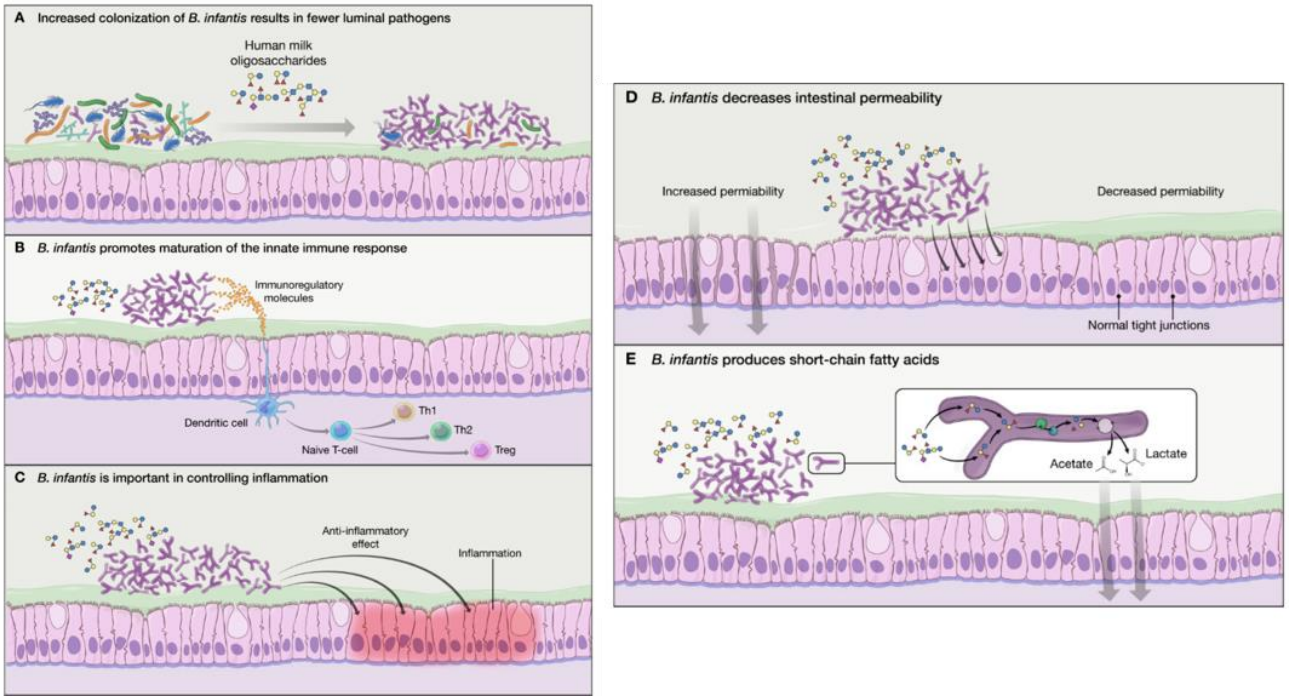

Figure 12: Effects of B. infantis on infant gut (64)

It should be noted that most infant formula are already supplemented with HMOs to promote the growth of *Bifidobacteria*, particularly, 2'-FL is added to enhance the growth of *B. infantis*. However, it has been observed that formula-fed infants often lack the necessary microbiota to successfully metabolise HMOs (65). As previously mentioned, this is due to an inherent deficit of Bifidobacteria, resulting in reduced utilisation of HMOs.

In contrast, a joint supplementation with *B. infantis* has proven more effective for the development of the microbiota. One study conducted a two-year follow-up of infants supplemented with *B. infantis* and observed that, after 6 and 12 months, the prevalence of *B. infantis* was significantly higher compared to controls supplemented with HMO only. In addition, a reduced amount of *Bacterroidaceae*, a family increased in ADHD, was noted. The research concluded that early *B.*

infantis supplementation contributes to the right development of the microbiota up to one year after the intervention (66).

Moreover, the administration of the prebiotics and probiotic does not have the same impact on infant gut. While 2'-FL raises the concentration of certain SCFA, direct administration *of B. infantis* increases the relative abundance of Bifidobacteria, further resembling the microbiota of a breastfed infant. It also leads to an increased production of acetate and general elevation of SCFA levels. Although more research is still needed, current evidence suggests that *B. infantis* supplementation may provide benefits over exclusive HMO supplementation (67).

A systematic review and meta-analysis of 67 articles, larger than the Cochrane review, concluded that *B. infantis* supports the maturation of the innate immune system and produces tryptophan metabolites, including ILA, which have anti-inflammatory properties. ILA stimulates anti-inflammatory cytokine release and is involved in gut barrier maintenance. In addition, short-chain fatty acid (SCFA) produced by *B infantis* have an impact on brain and adipose tissue, among others. For instance, by stimulating anti-inflammatory cytokines released by the intestinal cells, acetate is a SCFA crucial for the correct development of the immune system. Moreover*, B infantis* lower the faecal pH, protecting the gut from invasion and overgrowth of harmful bacteria (68).

Once the connection between Bifidobacterium and the immune system has been established, its effects must be evaluated at the CNS level. The study conducted by Bucells and colleagues proves the efficacy of *Lactobacillus* and *Bifidobacterium* supplementation in premature infants' neurodevelopment. A total of 233 premature children, born before 32 weeks and with a birth weight lower than 1500g, were included in the analysis. The investigation was carried out with preterm birth children as they are more likely to suffer from gut dysbiosis, specifically characterized by lower numbers of *Lactobacillus* and *Bifidobacterium*, and higher numbers of *Enterobacteriaceae*. This study is of great interest considering, as mentioned in previous sections, that dysbiosis is more frequent in children with ADHD. In addition, premature birth is also associated with this disorder. The results of the analysis after two years conclude that supplementation with *Bifidobacterias* combined with *Lactobacillus acidophilus* improved neurodevelopment. It is worth noting that, in this particular study, the positive effects cannot be directly attributed to *Bifidobacteria* due to co-administration with *Lactobacillus*. Nevertheless, multiple studies have assessed the isolated effect of *Bifidobacterium* on neurodevelopment, and the results are promising (69).

Additionally, the study by Knox et al evaluated the developmental impact of infants fed formula milk cultured with different strains of *Bifidobacterium* (70). All tested *Bifidobacterium* strains were found to improve both gut and brain barrier function. The intestinal epithelial barrier modulates interactions between the contents of the digestive tract and the body, and it is closely linked to the immune system, as increased permeability leads to heightened activation of the inflammatory response. Early stimulation of the immune system is beneficial for supporting its maturation, however, a loss of barrier integrity induces inflammatory stress and has a negative impact on the child (71). Significant differences have been observed between the microbiota of breastfed and formula-fed infants.

Bifidobacterium shows a lower relative abundance in formula-fed infants, and this decrease also correlates with increased systemic inflammation.

Supplementation with postbiotics from *Bifidobacterium* is not yet available because, although their impact on the immune system is proven, the mechanisms of action remain unclear. Nonetheless, there is sufficient evidence to suggest that direct supplementation with these bacteria may promote neurodevelopment by modulating neuroinflammation, a key factor in the development of ADHD (70).

Safety:

Probiotics are usually administered on children over 6 months due the fact that are alive organisms. However, *B. infantis* primarily colonizes the gut between the first and sixth month of life, in which it is considered a dominant species in a healthy microbiota, therefore an early supplementation is proposed. Multiple studies have evaluated the administration in premature infants and bacteremia caused by bifidobacteria has very rarely been reported. EFSA has given Bifidobacterium longum, which includes *B. infantis*, the status of Qualified Presumption of Safety (QPS). Put it differently, *B. infantis* strains are not associated with any human clinical disease *(68)*. In addition, there are commercial infant formula for newborns that contain *B. infantis*.

Stability and delivery approach:

Microencapsulation of *B. infantis* has been proposed as strategy to enhance its stability and ensure a sufficient concentration reaches the colon to exert a protective effect. To be effective, the bacteria must resist to bile and gastric acids and encapsulation improves resistance to these harsh conditions. Additionally, this formulation enables more controlled release. Capsules can be made of alginate or chitosan and lactic matrices. Moreover, co-encapsulation with prebiotics has also been investigated, as it further improves the bacterial survival rate by providing prebiotics as a substrate (62).

Commercial applications *of B. infantis:*

There is an increasing focus on the impact of the microbiota on the immune system and neurodevelopment. As a result, a growing number of companies are marketing probiotic formulations in this direction. For instance, ISTILOS TM *Bifidobacterium infantis* is a product by Novonesis indicated to ensure the correct development of the intestinal barrier and immune system in children (72). Additionally, *B. infantis* is already incorporated in some formula milks such as Nestle's NAN supremepro 1 milk, indicated for children from 0 to 6 months (73).

Limitations

Despite the increasing research on the impact of Bifidobacteria, there is still a lack of enquiry into specific *B. infantis* supplementation. *B.infantis* appears in several studies, however it is hardly ever evaluated independently, instead, it is usually combined with other probiotics, thus hindering to observe its real impact on the infant health. Nevertheless, current findings on Bifidobacteria, which include *B. infantis*, suggest that they may influence neurodevelopment and, consequently, the onset of ADHD.

7. Conclusion

A growing body of evidence indicates that the classical hypothesis of ADHD has proven insufficient to explain the development of the disorder. Hereditary genetic alterations contribute 70-80% and the remaining percentage is attributed to environmental factors. It should be noted that many of the environmental factors associated with the development of ADHD are additionally associated with dysbiosis. Dysbiosis is, therefore, a common feature among the factors associated with the onset of the disorder.

Interestingly, analyses of gut microbiota in children diagnose with ADHD reveal notable alterations. These modifications are mainly observed at the genus level, although at higher levels there are more discrepancies between studies. Thus, dysbiosis is found both in diagnosed children and in environmental factors associated with the development of the disorder. This led us to consider that there may be a close relationship between microbiota modifications and ADHD.

Additionally, the key role of early neuroinflammation in the development of ADHD has been observed. Neuroinflammation causes atypical brain function and is a consequence of chronic systemic inflammation. For this reason, the correct development of the child's immune system is fundamental and here the microbiota plays a determining role. Dysbiosis promotes systemic inflammation, contributing to neuroinflammation, and may ultimately lead to ADHD.

Breastfeeding is considered a protective factor against both ADHD and autoimmune diseases, suggesting that it also has an impact on the regulation of the immune system. Breast milk contains the HMOs which act as a substrate mainly for *Bifidobacterium infantis*. Currently, most commercial infant formulas contain these HMOs to enhance the growth of *B. infantis,* however, the effects differ from direct administration of the probiotic. When *B. infantis* is administered directly, the infant’s gut microbiota resembles more closely that of a breasted infant.

This *Bifidobacteria* not only contributes to the proper formation of the microbiota, but also it promotes the correct maturation of the immune system through the production of ILA and SCFA. It is worth noting that this probiotic is already marketed as an immunomodulator. In parallel, the impact of chronic inflammation in the development of ADHD is well demonstrated. Therefore, there is enough evidence to suggest that *B. infantis* could help prevent the onset of the disorder. Nonetheless, further research is still needed to support this hypothesis.

8. References

1. Sonuga-Barke EJ, Brandeis D, Cortese S, Daley D, Ferrin M, Holtmann M, et al. Nonpharmacological interventions for ADHD: Systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. American Journal of Psychiatry [Internet]. 2013 Mar 1 [cited 2024 Nov 4];170(3):275–89. Available from: https://psychiatryonline.org/doi/10.1176/appi.ajp.2012.12070991
2. Coghill D, Banaschewski T, Cortese S, Asherson P, Brandeis D, Buitelaar J, et al. The management of ADHD in children and adolescents: bringing evidence to the clinic: perspective from the European ADHD Guidelines Group (EAGG). Eur Child Adolesc Psychiatry [Internet]. 2021 Aug 1 [cited 2025 Jun 13];32(8):1337. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8532460/
3. Antolín BP, Gutiérrez-Abejón E, López SA, Llano JMA de. Tendencia del consumo de fármacos en el trastorno por déficit de atención e hiperactividad en niños y adolescentes (2010-2019): e202203033 [Internet]. Vol. 96, Revista Española de Salud Pública. 2022 [cited 2025 May 5]. p. 15 páginas-15 páginas. Available from: https://ojs.sanidad.gob.es/index.php/resp/article/view/308
4. Dam SA, Mostert JC, Szopinska-Tokov JW, Bloemendaal M, Amato M, Arias-Vasquez A. The Role of the Gut-Brain Axis in Attention-Deficit/Hyperactivity Disorder. Gastroenterol Clin North Am [Internet]. 2019 Sep 1 [cited 2024 Nov 4];48(3):407–31. Available from: https://pubmed.ncbi.nlm.nih.gov/31383279/
5. Epstein JN, Loren REA. Changes in the Definition of ADHD in DSM-5: Subtle but Important. Neuropsychiatry [Internet]. 2013 Oct [cited 2025 Apr 12];3(5):455. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3955126/
6. Posner J, Polanczyk G V., Sonuga-Barke E. Attention-deficit hyperactivity disorder. Lancet [Internet]. 2020 Feb 8 [cited 2024 Nov 11];395(10222):450. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7880081/
7. Demontis D, Walters GB, Athanasiadis G, Walters R, Therrien K, Nielsen TT, et al. Genome-wide analyses of ADHD identify 27 risk loci, refine the genetic architecture and implicate several cognitive domains. Nature Genetics 2023 55:2 [Internet]. 2023 Jan 26 [cited 2025 Apr 20];55(2):198–208. Available from: https://www.nature.com/articles/s41588-022-01285-8
8. MacDonald HJ, Kleppe R, Szigetvari PD, Haavik J. The dopamine hypothesis for ADHD: An evaluation of evidence accumulated from human studies and animal models. Front Psychiatry [Internet]. 2024 [cited 2025 Jun 8];15:1492126. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11604610/
9. Lewis N, Villani A, Lagopoulos J. Gut dysbiosis as a driver of neuroinflammation in attention-deficit/hyperactivity disorder: A review of current evidence. Neuroscience [Internet]. 2025 Mar 17 [cited 2025 Mar 22];569:298–321. Available from: https://www.sciencedirect.com/science/article/pii/S0306452225000338
10. Kumperscak HG, Gricar A, Ülen I, Micetic-Turk D. A Pilot Randomized Control Trial With the Probiotic Strain Lactobacillus rhamnosus GG (LGG) in ADHD: Children and Adolescents Report Better Health-Related Quality of Life. Front Psychiatry [Internet]. 2020 Mar 17 [cited 2024 Dec 15];11:181. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7092625/

11. Chronis-Tuscano A, Raggi VL, Clarke TL, Rooney ME, Diaz Y, Pian J. Associations between Maternal Attention-Deficit/Hyperactivity Disorder Symptoms and Parenting. J Abnorm Child Psychol [Internet]. 2008 Nov [cited 2025 Jun 19];36(8):1237. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3715319/
12. Hesselman S, Wikman A, Skoglund C, Kopp Kallner H, Skalkidou A, Sundström-Poromaa I, et al. Association of maternal attention deficit hyperactivity disorder and preterm birth: a cohort study. BJOG [Internet]. 2020 Nov 1 [cited 2025 May 5];127(12):1480–7. Available from: https://obgyn.onlinelibrary.wiley.com/doi/10.1111/1471-0528.16310
13. Zhou RY, Wang JJ, Sun JC, You Y, Ying JN, Han XM. Attention deficit hyperactivity disorder may be a highly inflammation and immune-associated disease (review). Mol Med Rep [Internet]. 2017 Oct 1 [cited 2025 Mar 22];16(4):5071–7. Available from: http://www.spandidos-publications.com/10.3892/mmr.2017.7228/abstract
14. Tseng PT, Yen CF, Chen YW, Stubbs B, Carvalho AF, Whiteley P, et al. Maternal breastfeeding and attention-deficit/hyperactivity disorder in children: a meta-analysis. Eur Child Adolesc Psychiatry [Internet]. 2019 Jan 30 [cited 2025 Feb 27];28(1):19–30. Available from: https://link.springer.com/article/10.1007/s00787-018-1182-4
15. Kwak MJ, Kim SH, Kim HH, Tanpure R, Kim JI, Jeon BH, et al. Psychobiotics and fecal microbial transplantation for autism and attention-deficit/hyperactivity disorder: microbiome modulation and therapeutic mechanisms. Front Cell Infect Microbiol [Internet]. 2023 [cited 2024 Nov 3];13:1238005. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10405178/
16. Du J, Fang L, Dong K, Zhou Z. Exploring the complex relationship between attention deficit hyperactivity disorder and the immune system: A bidirectional Mendelian randomization analysis. J Affect Disord [Internet]. 2025 Jan 15 [cited 2025 Mar 23];369:854–60. Available from: https://www.sciencedirect.com/science/article/pii/S0165032724017117
17. El Sehmawy AA, Abd Elaziz SY, Diab FEA, Al Anany MG, Elamir RY, Ibrahim HF, et al. Assessment of the natural killer cell, IFN- and IL-10 blood levels in children with attention-deficit/hyperactivity disorder. Egypt J Immunol [Internet]. 2023 Jul 1 [cited 2025 Jun 18];30(3):13–22. Available from: https://www.researchgate.net/publication/372343725_Assessment_of_the_natural_killer_cell_IFN-_and_IL-10_blood_levels_in_children_with_attention-deficithyperactivity_disorder
18. Misiak B, Wójta-Kempa M, Samochowiec J, Schiweck C, Aichholzer M, Reif A, et al. Peripheral blood inflammatory markers in patients with attention deficit/hyperactivity disorder (ADHD): A systematic review and meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry [Internet]. 2022 Aug 30 [cited 2025 Jun 9];118:110581. Available from: https://pubmed.ncbi.nlm.nih.gov/35660454/
19. Elsadek AE, Al-Shokary AH, Abdelghani WE, Kamal NM, Ibrahim AO, El-Shorbagy HH, et al. Serum Levels of Interleukin-6 and Tumor Necrosis Factor Alpha in Children With Attention-Deficit Hyperactivity Disorder. J Pediatr Neurosci [Internet]. 2021 Oct 1 [cited 2025 Mar 22];15(4):402. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8078629/
20. Soldati L, Deiber MP, Schockaert P, Köhl J, Bolmont M, Hasler R, et al. Sexually Transmitted Diseases and Attention-Deficit/Hyperactivity Disorder: A Systematic

Literature Review. J Psychiatr Pract [Internet]. 2024 Jul 26 [cited 2025 Jun 20];30(4):259. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11280443/
21. Kooij JJS, Bijlenga D, Salerno L, Jaeschke R, Bitter I, Balázs J, et al. Updated European Consensus Statement on diagnosis and treatment of adult ADHD. European Psychiatry [Internet]. 2019 Feb 1 [cited 2025 Jun 8];56(1):14–34. Available from: https://www.cambridge.org/core/journals/european-psychiatry/article/updated-european-consensus-statement-on-diagnosis-and-treatment-of-adult-adhd/707E2A36539213CF85EACCA576F47427
22. Martínez-Núñez B, Quintero J. Update the Multimodal Treatment of ADHD (MTA): twenty years of lessons. Actas Esp Psiquiatr [Internet]. 2019 Jan 1 [cited 2025 Apr 13];47(1):16–22. Available from: https://actaspsiquiatria.es/index.php/actas/article/view/175
23. Ministerio de sanidad servicios sociales e igualdad. GPC_574_TDAH_IACS_compl. 2017 [cited 2025 Jun 18]; Available from: https://portal.guiasalud.es/wp-content/uploads/2018/12/GPC_574_TDAH_IACS_compl.pdf
24. Fuster Nácher E, Pardo Corral M. REVISIONES EN FARMACOTERAPIA REVISIONES EN FARMACOTERAPIA Tratamiento farmacológico del trastorno por déficit de atención e hiperactividad (TDAH). 2021 [cited 2025 Jun 4];178–81. Available from: https://www.socesfar.es/wp-content/uploads/2022/01/AFTV19N3-06B-Revisiones-en-farmacoterapia.pdf
25. Xu X, Zhuo L, Zhang L, Peng H, Lyu Y, Sun H, et al. Dexmedetomidine alleviates host ADHD-like behaviors by reshaping the gut microbiota and reducing gut-brain inflammation. Psychiatry Res [Internet]. 2023 May 1 [cited 2025 May 20];323:115172. Available from: https://www.sciencedirect.com/science/article/pii/S0165178123001233?via%3Dihub
26. Jiang H yin, Zhou Y yue, Zhou G ling, Li Y chuan, Yuan J, Li X he, et al. Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. Behavioural Brain Research [Internet]. 2018 Jul 16 [cited 2024 Nov 28];347:408–13. Available from: https://pubmed.ncbi.nlm.nih.gov/29580894/
27. Wan L, Ge WR, Zhang S, Sun YL, Wang B, Yang G. Case-Control Study of the Effects of Gut Microbiota Composition on Neurotransmitter Metabolic Pathways in Children With Attention Deficit Hyperactivity Disorder. Front Neurosci [Internet]. 2020 Feb 18 [cited 2024 Nov 28];14:127. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7040164/
28. National Institute for Health and Care Excellence. Addendum to Clinical Guideline 72, Attention deficit hyperactivity disorder Clinical Guideline Addendum 72.1 Methods, evidence and recommendations. 2016 Feb [cited 2025 May 15]; Available from: https://www.nice.org.uk/guidance/ng87/evidence/dietary-interventions-pdf-4844210798
29. Elvin Dalaslan Ö, Çevi A, Çeli E, Topçu B. Evaluation of the relationship between the frequency of attention deficit, hyperactivity disorder symptoms and nutritional habits in children. Med J Malaysia [Internet]. 2024 Sep 1 [cited 2025 Feb 16];79(5):538–46. Available from: https://pubmed.ncbi.nlm.nih.gov/39352155/
30. Soto-Insuga V, Calleja ML, Prados M, Castaño C, Losada R, Ruiz-Falcó ML. Utilidad del hierro en el tratamiento del trastorno por déficit de atención e hiperactividad. An Pediatr (Engl Ed) [Internet]. 2013 Oct 1 [cited 2025 May 10];79(4):230–5. Available from: https://www.analesdepediatria.org/es-utilidad-del-hierro-el-tratamiento-articulo-S1695403313000805

31. World Health Organization. WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. 2020 [cited 2025 Jun 20]; Available from: https://iris.who.int/handle/10665/331505
32. Granero R, Pardo-Garrido A, Carpio-Toro IL, Ramírez-Coronel AA, Martínez-Suárez PC, Reivan-Ortiz GG. The role of iron and zinc in the treatment of adhd among children and adolescents: A systematic review of randomized clinical trials. Nutrients [Internet]. 2021 Nov 1 [cited 2025 Feb 16];13(11):4059. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8618748/
33. Arnold LE, Disilvestro RA, Bozzolo D, Bozzolo H, Crowl L, Fernandez S, et al. Zinc for Attention-Deficit/Hyperactivity Disorder: Placebo-Controlled Double-Blind Pilot Trial Alone and Combined with Amphetamine. J Child Adolesc Psychopharmacol [Internet]. 2011 Feb 1 [cited 2025 Feb 18];21(1):1. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3037197/
34. Zamora J, Velásquez A, Troncoso L, Barra P, Guajardo K, Castillo-Duran C. ARCHIVOS LATINOAMERICANOS DE NUTRICIÓN Órgano Oficial de la Sociedad Latinoamericana de Nutrición. 2011;61.
35. Noorazar SG, Malek A, Aghaei SM, Yasamineh N, Kalejahi P. The efficacy of zinc augmentation in children with attention deficit hyperactivity disorder under treatment with methylphenidate: A randomized controlled trial. Asian J Psychiatr [Internet]. 2020 Feb 1 [cited 2025 Feb 17];48:101868. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1876201819308019
36. Dicks LMT. Gut Bacteria and Neurotransmitters. Microorganisms [Internet]. 2022 Sep 1 [cited 2024 Nov 1];10(9):1838. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9504309/
37. Yang LL, Stiernborg M, Skott E, Xu J, Wu Y, Landberg R, et al. Effects of a Synbiotic on Plasma Immune Activity Markers and Short-Chain Fatty Acids in Children and Adults with ADHD—A Randomized Controlled Trial. Nutrients [Internet]. 2023 Mar 1 [cited 2025 Feb 4];15(5):1293. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10004766/
38. Góralczyk-Bińkowska A, Szmajda-Krygier D, Kozłowska E. The Microbiota–Gut–Brain Axis in Psychiatric Disorders. Int J Mol Sci [Internet]. 2022 Oct 1 [cited 2024 Nov 1];23(19):11245. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9570195/
39. Cickovski T, Mathee K, Aguirre G, Tatke G, Hermida A, Narasimhan G, et al. Attention Deficit Hyperactivity Disorder (ADHD) and the gut microbiome: An ecological perspective. PLoS One [Internet]. 2023 Aug 1 [cited 2024 Nov 9];18(8):e0273890. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10437823/
40. Zang Y, Lai X, Li C, Ding D, Wang Y, Zhu Y. The Role of Gut Microbiota in Various Neurological and Psychiatric Disorders—An Evidence Mapping Based on Quantified Evidence. Mediators Inflamm [Internet]. 2023 [cited 2025 May 14];2023:5127157. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9936509/
41. Gkougka D, Mitropoulos K, Tzanakaki G, Panagouli E, Psaltopoulou T, Thomaidis L, et al. Gut microbiome and attention deficit/hyperactivity disorder: a systematic review. Pediatric Research 2022 92:6 [Internet]. 2022 Mar 30 [cited 2025 May 15];92(6):1507–19. Available from: https://www.nature.com/articles/s41390-022-02027-6
42. Martín-Carrasco M. Psicobióticos y trastornos mentales. Ponencia realizada al V Workshop INSA Cátedra UB-DANONE “Prebióticos y microbiota en una dieta saludable y sostenible.” Santa Coloma de Gramanet; 2024 May.

43. Aarts E, Ederveen THA, Naaijen J, Zwiers MP, Boekhorst J, Timmerman HM, et al. Gut microbiome in ADHD and its relation to neural reward anticipation. PLoS One [Internet]. 2017 Sep 1 [cited 2024 Nov 11];12(9):e0183509. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5581161/
44. Prehn-Kristensen A, Zimmermann A, Tittmann L, Lieb W, Schreiber S, Baving L, et al. Reduced microbiome alpha diversity in young patients with ADHD. PLoS One [Internet]. 2018 Jul 1 [cited 2024 Nov 27];13(7):e0200728. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6042771/
45. Wang LJ, Yang CY, Chou WJ, Lee MJ, Chou MC, Kuo HC, et al. Gut microbiota and dietary patterns in children with attention-deficit/hyperactivity disorder. Eur Child Adolesc Psychiatry [Internet]. 2020 Mar 1 [cited 2024 Nov 28];29(3):287–97. Available from: https://link.springer.com/article/10.1007/s00787-019-01352-2
46. Cheng S, Han B, Ding M, Wen Y, Ma M, Zhang L, et al. Identifying psychiatric disorder-associated gut microbiota using microbiota-related gene set enrichment analysis. Brief Bioinform [Internet]. 2020 May 18 [cited 2024 Nov 28];21(3):1016–22. Available from: https://dx.doi.org/10.1093/bib/bbz034
47. Dezfouli MA, Rashidi SK, Yazdanfar N, Khalili H, Goudarzi M, Saadi A, et al. The emerging roles of neuroactive components produced by gut microbiota. Molecular Biology Reports 2024 52:1 [Internet]. 2024 Nov 21 [cited 2025 Mar 18];52(1):1–18. Available from: https://link.springer.com/article/10.1007/s11033-024-10097-4
48. Eiche TP, Mohajeri MH. Overlapping Mechanisms of Action of Brain-Active Bacteria and Bacterial Metabolites in the Pathogenesis of Common Brain Diseases. Nutrients [Internet]. 2022 Jul 1 [cited 2025 May 15];14(13):2661. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9267981/
49. Wang Q, Yang Q, Liu X. The microbiota–gut–brain axis and neurodevelopmental disorders. Protein Cell [Internet]. 2023 Oct 1 [cited 2025 May 21];14(10):762. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10599644/
50. Rusch JA, Layden BT, Dugas LR, Vaudry H, Zhou M, Raber J. Signalling cognition: the gut microbiota and hypothalamic-pituitary-adrenal axis. Front Endocrinol (Lausanne) [Internet]. 2023 [cited 2024 Nov 1];14:1130689. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10316519/
51. Okburan G, Kızıler S. Human milk oligosaccharides as prebiotics. Pediatr Neonatol [Internet]. 2023 May 1 [cited 2025 Mar 16];64(3):231–8. Available from: https://www.pediatr-neonatol.com/action/showFullText?pii=S187595722200273X
52. Martín-Ramos S, Domínguez-Aurrecoechea B, García Vera C, Lorente García Mauriño AM, Sánchez Almeida E, Solís-Sánchez G. Lactancia materna en España y factores relacionados con su instauración y mantenimiento: estudio LAyDI (PAPenRed). Aten Primaria [Internet]. 2023 Jan 1 [cited 2025 Mar 6];56(1):102772. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10520303/
53. World Health Organization. LA LACTANCIA MATERNA. 2016 [cited 2025 May 23]; Available from: https://www.paho.org/sites/default/files/LactanciaMaternaEnSigloXXI-April15.pdf
54. Kim JH, Lee SW, Lee JE, Ha EK, Han MY, Lee E. Breastmilk feeding during the first 4 to 6 months of age and childhood disease burden until 10 years of age. Nutrients [Internet]. 2021 Aug 1 [cited 2025 Feb 27];13(8):2825. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8400284/

55. Shamberger R. Attention-Deficit Disorder Associated with Breast-Feeding: A Brief Report. J Am Coll Nutr [Internet]. 2012 Aug 1 [cited 2025 Feb 16];31(4):239–42. Available from: https://www.tandfonline.com/doi/abs/10.1080/07315724.2012.10720422
56. Davis EC, Castagna VP, Sela DA, Hillard MA, Lindberg S, Mantis NJ, et al. Gut Microbiome and Breast-feeding: Implications for Early Immune Regulation. J Allergy Clin Immunol [Internet]. 2022 Sep 1 [cited 2025 Feb 6];150(3):523. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9463492/
57. Pärtty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatric Research 2015 77:6 [Internet]. 2015 Mar 11 [cited 2024 Dec 15];77(6):823–8. Available from: https://www.nature.com/articles/pr201551
58. Sun W, Tao L, Qian C, Xue P, Tong X, Yang L, et al. Human milk oligosaccharides and the association with microbiota in colostrum: a pilot study. Arch Microbiol [Internet]. 2024 Feb 1 [cited 2025 May 23];206(2):58. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10774193/
59. Pucci N, Ujčič-Voortman J, Verhoeff AP, Mende DR. Priority effects, nutrition and milk glycan-metabolic potential drive Bifidobacterium longum subspecies dynamics in the infant gut microbiome. PeerJ [Internet]. 2025 Jan 21 [cited 2025 Mar 15];13(1):e18602. Available from: https://peerj.com/articles/18602
60. Selma-Royo M, Calvo Lerma J, Cortés-Macías E, Collado MC. Human milk microbiome: From actual knowledge to future perspective. Semin Perinatol [Internet]. 2021 Oct 1 [cited 2025 Mar 6];45(6):151450. Available from: https://www.sciencedirect.com/science/article/pii/S0146000521000641?via%3Dihub
61. Henrick BM, Rodriguez L, Lakshmikanth T, Pou C, Henckel E, Arzoomand A, et al. Bifidobacteria-mediated immune system imprinting early in life. Cell [Internet]. 2021 Jul 22 [cited 2025 May 26];184(15):3884-3898.e11. Available from: https://www.cell.com/action/showFullText?pii=S0092867421006607
62. Dargenio VN, Cristofori F, Brindicci VF, Schettini F, Dargenio C, Castellaneta SP, et al. Impact of Bifidobacterium longum Subspecies infantis on Pediatric Gut Health and Nutrition: Current Evidence and Future Directions. Nutrients [Internet]. 2024 Oct 1 [cited 2025 Jun 2];16(20):3510. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC11510697/
63. Laursen MF, Sakanaka M, von Burg N, Mörbe U, Andersen D, Moll JM, et al. Bifidobacterium species associated with breastfeeding produce aromatic lactic acids in the infant gut. Nature Microbiology 2021 6:11 [Internet]. 2021 Oct 21 [cited 2025 Feb 19];6(11):1367–82. Available from: https://www.nature.com/articles/s41564-021-00970-4
64. Chichlowski M, Shah N, Wampler JL, Wu SS, Vanderhoof JA. Bifidobacterium longum Subspecies infantis (B. infantis) in Pediatric Nutrition: Current State of Knowledge. Nutrients [Internet]. 2020 Jun 1 [cited 2025 Jun 12];12(6):1581. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7352178/
65. De Bruyn F, James K, Cottenet G, Dominick M, Katja J. Combining Bifidobacterium longum subsp. infantis and human milk oligosaccharides synergistically increases short chain fatty acid production ex vivo. Communications Biology 2024 7:1 [Internet]. 2024 Aug 4 [cited 2025 Jun 3];7(1):1–12. Available from: https://www.nature.com/articles/s42003-024-06628-1

66. O'Brien CE, Meier AK, Cernioglo K, Mitchell RD, Casaburi G, Frese SA, et al. Early probiotic supplementation with B. infantis in breastfed infants leads to persistent colonization at 1 year. Pediatr Res [Internet]. 2021 Feb 1 [cited 2025 May 29];91(3):627. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8460680/
67. Wang M, Monaco MH, Daniels VC, Hirvonen J, Jensen HM, Ouwehand AC, et al. Individual and Combined Effects of 2'-Fucosyllactose and Bifidobacterium longum subsp. infantis on the Gut Microbiota Composition of Piglets. J Nutr [Internet]. 2025 Feb 1 [cited 2025 Jun 3];155(2):509–22. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022316624012495
68. Batta VK, Rao SC, Patole SK. Bifidobacterium infantis as a probiotic in preterm infants: a systematic review and meta-analysis. Pediatr Res [Internet]. 2023 Dec 1 [cited 2025 Apr 20];94(6):1887. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10665187/
69. Baucells BJ, Sebastiani G, Herrero-Aizpurua L, Andreu-Fernández V, Navarro-Tapia E, García-Algar O, et al. Effectiveness of a probiotic combination on the neurodevelopment of the very premature infant. Scientific Reports 2023 13:1 [Internet]. 2023 Jun 26 [cited 2025 May 29];13(1):1–9. Available from: https://www.nature.com/articles/s41598-023-37393-6
70. Knox EG, Sánchez-Díaz P, Buttimer C, Leigh SJ, Vlckova K, Hill C, et al. Bifidobacterium fermentation with infant formulas is associated with benefits for gut and brain barrier function. J Funct Foods. 2025 Feb 1;125:106661.
71. Charton E, Bourgeois A, Bellanger A, Le-Gouar Y, Dahirel P, Romé V, et al. Infant nutrition affects the microbiota-gut-brain axis: Comparison of human milk vs. infant formula feeding in the piglet model. Front Nutr [Internet]. 2022 Sep 21 [cited 2025 Jun 12];9:976042. Available from: https://www.frontiersin.org/journals/nutrition/articles/10.3389/fnut.2022.976042/full
72. ISTILOS™ B. infantis [Internet]. [cited 2025 Jun 12]. Available from: https://www.chr-hansen.com/es/human-health-and-probiotics/our-probiotic-strains/istilos
73. Nestlé NAN Supreme Pro 1: leche de inicio | Nestlé Bebé [Internet]. [cited 2025 Jun 12]. Available from: https://www.nestlebebe.es/productos-infantiles/leches-de-formula/leches-inicio/nan-supreme-1

ANNEX:

In order to gain a better understanding of ADHD and to complement the theoretical research, several interviews were conducted with different specialists in microbiota and/or ADHD.
Among the participants are a Danone researcher, a paediatrician, a child psychiatrist, a gastroenterologist and a representative of a laboratory specialising in probiotic production. Some of the interviews have been transcribed, while others could not be recorded.

For confidentiality reasons, the interview transcripts are not included in this publicly available version.