Impact of Bioactive Lipids on Gut microbiota

Ana Sofia Salsinha[a,b], Manuela Pintado[a,*]

[a]Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua de Diogo Botelho, 1327, 4169-005, Porto, Portugal

[b]Instituto de Investigação e Inovação em Saúde and Instituto de Biologia Molecular e Celular (IBMC), Rua Alfredo Allen, 208 4200-135, Porto, Portugal

*Corresponding author:

mpintado@ucp.pt

Escola Superior de Biotecnologia 11 Universidade Católica Portuguesa | Porto

Rua de Diogo Botelho, 1327 12 4169-005 Porto, Portugal

Tel.: +351 225580097

Mobile: +351 9333095043

Abstract

Different bacteria cohabit different sites of the human body, with special relevance on the mutualistic collaboration responsible for the maintenance of the gastrointestinal tract's homeostasis. Diversification and composition of the microbiota are influenced by several factors, including diet. Several beneficial effects have been linked with microbiota modulation and its dysregulation have been associated with several pathologies. Lipids are essential elements in gut permeability, being important in the modulation of the gut microbiome. Bioactive lipids like endocannabinoids, short-chain fatty acids (SCFAs) and omega-3 fatty acids have been associated with microbiota modulation. The endocannabinoid system is involved in modulation of gastric emptying, gastrointestinal motility and inflammation. SCFAs are a source of energy to the colonic epithelium and maintain the integrity of the epithelial barrier. SCFAs also interact with the blood-brain barrier being able to modulate brain function. Omega-3 potentially restore the gut dysbiosis that is encountered in several pathologies. Thus, all these bioactive lipids have important functions in gut dysbiosis observed in pathologies like inflammatory bowel disease, obesity, Alzheimer's disease, multiple sclerosis and Parkinson's disease.

Abbreviations

α-linolenic acid (ALA); α-synuclein protein (αSyn); 2-Arachidonoylglycerol (2-AG); 12-hydroxyeicosatetraenoic acid (12-HETE); Blood-brain barrier (BBB); Cannabinoid receptor 1 (CB1); Central Nervous System (CNS); Docosahexaenoic acid (DHA); Eicosapentaenoic acid (EPA); Enteric nervous system (ENS); Gamma-aminobutyric acid (GABA); G-protein coupled membrane receptors (GPR); Inflammatory bowel disease (IBD); Lipopolysaccharide (LPS); Medium-chain fatty acid (MCFA); Multiple Sclerosis (MS); Mu-opioid receptors (MOR); N-acylethanolamine (NAE); N-arachidonoylethanolamine (AEA); Parkinson's disease (PD); Peroxisome proliferator-activated receptor (PPAR); Polyunsaturated fatty acids (PUFAs); Relapsing remitting multiple sclerosis (RRMS); Short-chain fatty acids (SCFAs); Wild-type (WT)

1. Introduction

The total number of bacteria in a standard adult man of 70 kg is estimated to be $3.8x10^{13}$, being the number and diversity of microorganisms greatly modulated by physiological and environmental factors (Belizário & Faintuch, 2018; Sender et al., 2016). Although the gut microbiota also includes archaea, viruses and fungi, more than 99% of the microbial genes detected in the gut are bacterial genes (Cani et al., 2016). Importantly, throughout the years, the evolution of *Homo sapiens* has been closely connected to mutualistic collaboration among the diversity of bacteria that cohabit the different sites of the human body, with special focus on the gastrointestinal tract. In the gut, most bacteria are adherent to mucus, important to form the outer and inner physical barrier.

More than 90% of the bacterial species comprising the human microbiome belong to 4 major phyla: Firmicutes, which account for 65% of gut microorganisms, Bacteroidetes (16%), Actinobacteria (9%) and Proteobacteria (5%). The Firmicutes phylum is mainly comprised of bacteria from Bacilli (*e.g*. *Lactobacillus*) and Clostridia classes, which are Gram-positive bacteria. While Bacilli are obligate or facultative aerobes, Clostridia are anaerobic. On the other hand, the Bacteroidetes phylum corresponds to Gram-negative, non-spore-forming, anaerobic bacteria which can tolerate the presence of oxygen but cannot use it for growth. Regarding Actinobacteria phylum, comprising for example the *Bifidobacterium* genus, they are Gram-positive, with multiple branching rods, non-motile, non-spore-forming and anaerobic bacteria. Lastly, Proteobacteria, *e.g. Escherichia, Klebsiella, Enterobacter* genus, are aerobic or facultative anaerobic, Gram-negative, non-spore-forming rods bacteria known to be present in the intestinal tract of all vertebrates. Importantly, such organisms are transmitted to babies mainly via mother's milk, which is rich in *Bifidobacterium* and *Lactobacillus* (as reviewed by (Belizário & Faintuch, 2018)). Indeed, the establishment of the gut microbiota occurs early in life, starting from a low level of diversity, increasing throughout the years and reaching a complexity comparable to the adult microbiota by 3 to 5 years of age (Cani et al., 2016). Later, in adults, the majority of bacteria belongs to the genera *Bacteroides* (Bacteroidetes phylum), *Parabacteroides* (Bacteroidetes phylum) and *Clostridium* (Firmicutes phylum). Nevertheless, each site of the gastrointestinal tract presents a distinctive microbiota (Belizário & Faintuch, 2018). In fact, the diversification and composition of the microbiota are influenced by several factors, such as perinatal features (mode of delivery: caesarian section or vaginal delivery), nutrition and weaning. As mentioned, environmental and host-specific factors, such as genotype, age and gender, as well as habitat and most importantly diet, are determinants to define the host's microbiome (Belizário & Faintuch, 2018; Cani et al., 2016).

1.1. Importance of Gut microbiota on host's homeostasis

Gut microbiota's importance lies in its role in maintaining the host's normal homeostasis through three major functions: protection against pathogen colonization, achieved through nutrient competition and production of antimicrobial agents (*e.g.* hydrogen peroxide, acidophylin, acidolin, lactallin, etc.); stimulation of innate immunity and restriction of toxins production and penetration of pathogenic microorganisms into gut tissues; promotion of nutrient absorption through indigestible dietary fibers or tri/tetrasaccharides metabolization to monosaccharides ultimately producing B-group vitamins (Russo et al., 2017). Besides, the gut microbiota also interacts with

enteroendocrine cells, produces vitamins, steroid hormones and neurotransmitters such as gamma-aminobutyric acid (GABA) and serotonin (Melbye et al., 2019). Specifically, some species, namely *Akkermansia muciniphila* and *Bifidobacterium* spp. are believed to be especially relevant since they mediate multiple interactions between microorganisms (Cani et al., 2016).

Considering the importance of gut microbiota in the host's homeostasis, the development and use of prebiotics and probiotics have been widely assessed throughout the years. They are relevant since they can change the composition of the gut microbiota consequently being able to induce beneficial effects. Prebiotics are nondigestible food ingredients that stimulate the growth and/or activity of gut bacteria, benefiting the health of the host. On the other hand, probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts (Cani et al., 2016). As mentioned, the importance of gut microbiota in the host's homeostasis has been widely assessed and its fundamental role in the development and therapeutical approach of several diseases has been determined. For instance, key roles for the gut microbiota and its metabolites have been attributed regarding glucose metabolism. Indeed, by using prebiotics and/ or probiotics microbiota remodeling has been associated with improved glucose metabolism in subjects with type 2 diabetes (Abot et al., 2021; Cani & Knauf, 2016; Gibson et al., 2017; Rastelli et al., 2019). Nonetheless, several beneficial effects have been associated with microbiota modulation. In fact, microbiota dysregulation has been connected with several pathologies and the important role of bioactive lipids in such processes has been unraveled, as is going to be discussed in this chapter.

1.2. Gut Dysbiosis

Gut dysbiosis is a persistent imbalance of gut microbial species abundance, caused by a loss of microbe species richness and increased interindividual variability. Such alteration results in impaired gut barrier function as well as inflammatory cells activation. Therefore, gut dysbiosis has been associated with the onset of several pathological diseases not only involving the gastrointestinal system but also other organs. Among the diseases associated with such problems, there is inflammatory bowel disease (IBD), irritable bowel syndrome, diabetes, obesity, cancer, cardiovascular and central nervous system (CNS) disorders, namely neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease (AD) and Multiple sclerosis (MS) (Baptista et al., 2020; Belizário & Faintuch, 2018). Shortly, in several of the mentioned diseases where gut dysbiosis occurs, a strong inflammatory component has been associated with it. In general terms, in healthy individuals, anti-inflammatory species - *e.g. Faecalibacterium prausnitzii* - are predominantly present. In contrast, in IBD, PD, Crohn's disease and obesity, for example, there is the presence of potential proinflammatory bacteria such as *Bacteroides, Ruminococcus gnavus, Verrucomicrobia,* associated with the production of inflammatory factors (Belizário & Faintuch, 2018; Henke et al., 2019; Lin et al., 2019; Tseng & Wu, 2019). Nevertheless, lately has been suggested that it is not specific modulation of a single bacterium the source of pathology development instead, general alterations to the ecosystems seem to be responsible.

2. Bioactive lipid’s role on Gut microbiota function

Lipids are the major constituents of cell membranes thus, being essential elements in gut permeability. Due to this role, they are important in the modulation of the gut microbiome. Besides their structural role, lipids regulate multiple cell functions through intercellular and intracellular signaling mediators present both in the brain and the enteric system. These lipids are known as bioactive lipids. These molecules are released into the bloodstream, migrating to distant organs through the gut-brain axis. Importantly, multiple bioactive lipids exert either pro- or anti-inflammatory actions on the gut microbiome, influencing several important processes such as immune regulation, inflammation and homeostasis (as reviewed by (Baptista et al., 2020)). For instance, the enteric nervous system (ENS) has been considered an important target to treat type 2 diabetes. This is related to the fact that type 2 diabetes leads to duodenal hypercontractility which results in aberrant signaling from the afferent nerves to the hypothalamus, ultimately resulting in systemic insulin resistance. There has been an association between microbiota role on ENS and bioactive lipids; indeed, microbiota bacteria directly modulate enteric neurons or indirectly through the release of bioactive lipids, such as short-chain fatty acids (SCFAs) (Cani & Knauf, 2016). Moreover, the improvement of the diabetic state was associated with an increase in the levels of 12-hydroxyeicosatetraenoic acid (12-HETE). This bioactive lipid, which is derived from arachidonic acid (AA), is considered a second messenger, which is responsible to transmit signals from activated mu-opioid receptors (MOR) and subsequently increases the activity of potassium channels to inhibit neurotransmitter release in neurons. It was demonstrated that prebiotic supplementation (with FOS) increases the release of 12-HETE in the colon of mice and that such release is associated with a decrease of duodenal contraction through 12S-HETE/MOR and peroxisome proliferator-activated receptor (PPAR)-γ signaling (Abot et al., 2021).

Other relevant roles of bioactive lipids in microbiota modulation and consequently in pathology development have been reported. Thus, in the next sections the more relevant bioactive lipids in the context of gut microbiota function and modulation and disease progression are going to be discussed.

2.1.Endocannabinoid system

N-arachidonoylethanolamine (AEA) is a member of the N-acylethanolamine (NAE) family, a large group of bioactive lipids. 2-Arachidonoylglycerol (2-AG) is another member of the same endocannabinoid family which was identified in the intestine. Although further members of the endocannabinoid family have been identified and other AA derivatives have been shown to interact with endocannabinoid receptors, AEA and 2-AG are the most studied. AEA and 2-AG are synthesized from AA through enzymatic activation by multiple pathways in the membrane of different cell types such as neurons, adipocytes, and skeletal muscle cells, in response to increased intracellular Ca^{2+} concentration, membrane depolarization, and/or receptor stimulation (Bisogno et al., 2021). These bioactive lipids comprise the endocannabinoid system. Such system is widely known to be mediated by cannabinoid receptor 1 (CB1) and CB2, which are G-protein coupled membrane receptors (GPR). Besides, endocannabinoids also interact with PPAR-α and PPAR-γ as well as with GPR-55. In addition, molecules that structurally resemble the mentioned endocannabinoids - endocannabinoid analogs or related bioactive lipids- have been shown to be able to mediate or interfere with the endocannabinoid response without activating the endocannabinoid receptors, including lipids that belong to the acylglycerol family such as palmitoyl-glycerol (2-PG) and

oleoylglycerol (2-OG) (Cani et al., 2016). Indeed, endocannabinoids are greatly produced in organs that contribute to the regulation of energy homeostasis, such as the brain, liver, adipose tissue, muscles and pancreas. Furthermore, the endocannabinoid system is involved in gut physiology through modulation of gastric emptying, gastrointestinal motility and inflammation. Interestingly, among the factors involved in energy balance, the gut microbiota has a crucial role. Evidence suggests that the gut microbiota contributes to host metabolism by communicating with adipose tissue, primarily by regulating fat storage (Cani et al., 2016).

Such observations suggest *a priori* a link between microbiota and the endocannabinoid system; indeed a study has demonstrated that oral administration of a *Lactobacillus acidophilus* strain induced the expression of cannabinoid and μ-opioid receptors - previously demonstrated to have anti-inflammatory functions in several experimental models of colitis - in intestinal cells, and mediated analgesic functions in the gut by reducing abdominal pain in a rat model of chronic colonic hypersensitivity, mimicking irritable bowel syndrome (Rousseaux et al., 2007). Moreover, endocannabinoid system has been suggested as a possible link between microbiota and adipose tissue, developing an important role in obesity (Cani et al., 2016). The link between the endocannabinoid system and gut microbiota dysbiosis is going to be discussed in this chapter for each of the relevant selected pathologies.

2.2.Short-chain fatty acids

SCFAs are carboxylic acids containing 2 to 5 carbon atoms and are considered metabolites that are produced by bacterial fermentation of non-digestible carbohydrates such as dietary fibers and resistant starch, in the proximal colon. Importantly, they have been shown to have immunomodulatory effects. In fact, they can promote anti-inflammatory effects. Interestingly, the type and number of fibers consumed affect the composition of the gut microbiota and consequently the type and amount of SCFAs produced. The most abundant SCFAs are acetate (C2), propionate (C3) and butyrate (C4) being produced by bacterial species within the phyla Firmicutes, Bacteroidetes and Actinobacteria. SCFAs importance lies in the fact that they are a source of energy to the colonic epithelium and maintain the integrity of the epithelial barrier by regulating mucus production and tight junction expression (as reviewed by (Melbye et al., 2019)). Importantly, SCFAs also interact with the blood-brain barrier (BBB) being able to cross it and consequently modulate brain function. It is suggested that SCFAs trigger mucin production and tight junction synthesis, which strengthen the intestinal epithelial barrier ultimately leading to a reduced passage of toxic and proinflammatory substances across the epithelial barrier. Importantly, the presence of SCFAs also modulates T-cell differentiation towards regulatory subtypes (*i.e* Treg cells) and suppresses proinflammatory Th17 and Th1 cells. It is the balance between such cells that drives pathogenic or protective responses in other tissues, such as the CNS and modulates autoimmunity. Especially the generation of Th17 cells and their cytokines have been closely associated with gut microbiota. Both Th1 and Th17 cells, can cross the BBB and cause CNS inflammation after activation through microglia modulation. Proteins claudin-5 and occludin are tight junction proteins important in BBB permeability. Increased BBB permeability has been associated with reduced expression of these proteins along with changes in gut microbiota of mice. Importantly, butyrate was shown to upregulate these proteins, thereby restoring the BBB permeability in mice (Melbye et al., 2019). In fact, SCFAs' gastrointestinal levels are associated with CNS disorders,

namely AD, suggesting their important role on gut-brain communication (Bisogno et al., 2021). Moreover, in inflammatory diseases, such as IBD and obesity, SCFAs develop an important role since they can also act as signaling molecules to activate GPR receptors - GPR41 and GPR43 - activating signaling cascades that control immune functions (Parada Venegas et al., 2019; Tseng & Wu, 2019).

Furthermore, gut microbiota also seems to play a crucial role in the development and functionality of the CNS immune system, specifically in microglia modulation. Interestingly, microglia from specific pathogen-free mice shows normal maturation and function, while non-colonized young germ-free mice exhibit stunted microglia under homeostatic conditions, suggesting an important role of gut microbiota in this process. Moreover, the oral application of SCFAs (a mixture of acetate, propionate and butyrate) was found sufficient to induce maturation of microglia in germ-free mice (Silva et al., 2020). Nevertheless, the mechanisms involved in this modulation remain unknown.

Additionally, SCFAs improve gut health by maintaining intestinal barrier integrity, mucus production and protection against inflammation. As mentioned, it is known that SCFAs bind to GPRs, namely GPR43, GPR41, GPR109a/HCAR2 (hydrocarboxylic acid receptor) and GPR164, present in cells from the intestinal mucosa, immune and nervous system. The binding of SCFAs to their receptors on enteroendocrine cells, results in stimulated secretion of glucagon-like peptide 1 and peptide YY, while signaling in β-pancreatic cells leads to increased insulin secretion. Effects on brown adipose tissue activation, regulation of liver mitochondrial function, whole-body energy homeostasis, and control of appetite and sleep have also been attributed to SCFAs (Silva et al., 2020).

2.3.Polyunsaturated fatty acids: Omega-3

Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs), being eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) the two main bioactive forms in humans. They are synthesized from the dietary precursor, α-linolenic acid (ALA). They are important regulators of inflammatory processes, being recognized as having an anti-inflammatory role. Thus, possessing health benefits against different chronic degenerative diseases, such as cardiovascular diseases, rheumatoid arthritis, IBD, cognitive disorders, depression, cancer and obesity (Costantini et al., 2017). Omega-3 and omega-6 PUFAs are suggested to have antagonistic roles in inflammatory response: omega-6 have a proinflammatory role, by acting as a precursor to inflammatory eicosanoids; omega-3 present anti-inflammatory actions competing with the same enzymatic pathway. In a similar way, these PUFAs seem to have opposing effects on intestinal homeostasis (Robertson, Seira Oriach, Murphy, Moloney, Cryan, Dinan, Paul Ross, et al., 2017; Robertson, Seira Oriach, Murphy, Moloney, Cryan, Dinan, Ross, et al., 2017).

Contrarily to SCFAs, the impact of omega-3 on the gut microbiota is poorly defined. Very few studies were performed on adults, nevertheless, they showed promising results as there were some common changes in the gut microbiota after omega-3 PUFA supplementation: decrease in *Faecalibacterium*, which is often associated with an increase in Bacteroidetes and butyrate-producing bacteria. Moreover, some in vivo studies have shown that the interplay between gut microbiota, omega-3 fatty acids and

immune response allows the maintenance of intestinal wall integrity and interaction with host immune cells (Costantini et al., 2017).

In summary, omega-3's potential action in gut microbiota lies in restoring the dysbiosis that is encountered in several pathologies. Indeed. the dysbiosis of the Firmicutes/Bacteroidetes ratio is associated with several conditions, such as weight gain and obesity, insulin resistance, high-fat diet (HFD), gut permeability, IBD, and depression. Omega-3 is able to reverse this condition by restoring the Firmicutes/Bacteroidetes ratio and increasing Lachnospiraceae bacteria, both are importantly associated with increased production of the anti-inflammatory SCFA butyrate. In addition. omega-3 PUFAs increase lipopolysaccharide (LPS)-suppressing bacteria, Bifidobacteria and decrease LPS-producing bacteria, Enterobacteria, suppressing endotoxemia responsible for a low-grade systemic inflammation (as reviewed by (Costantini et al., 2017)). Thus, they are considered prebiotics in some pathologies.

In conclusion, the positive effects of omega-3 fatty acids in gut microbiota and associated pathologies are closely connected to their anti-inflammatory capacity, nonetheless and despite promising results, there is still a lack of knowledge regarding this theme and further studies are warranted.

3. Gut microbiota effect on pathology development and the role of bioactive lipids

3.1. Inflammatory Bowel Disease

Inflammatory bowel disease is described as a chronic and relapsing inflammatory disorder of the gut, comprising both Crohn's Disease and ulcerative colitis. The incidence of both diseases has been increasing worldwide, especially in developing/Western countries, suggesting that indeed dietary patterns may play a role in such pathology. In fact, several studies have been associating dietary components, namely dietary intake of fruits, vegetables, dairy products, iron and vitamin D, to the etiology of IBD (Mozaffari et al., 2020). Nevertheless, IBD is a result of an interplay of different factors, such as genetic, immunologic, microbial and environmental factors (Parada Venegas et al., 2019). The most consistent observation in IBD is reduced bacterial diversity, a decrease of Firmicutes, and an increase of Proteobacteria. Moreover, it was observed that in Crohn's disease, dysbiosis is characterized by the loss of intestinal bacteria from the Firmicutes phylum, including *Faecalibacterium prausnitzii* (Matsuoka & Kanai, 2015), which are considered highly relevant butyrate-producing bacterium in the gut. It has been observed that stress-induced reduction of *Lactobacillus reuteri*, a specific immunomodulatory species of bacteria, leads to an increased proinflammatory gene expression and monocyte differentiation (Russo et al., 2017). Moreover, the probiotic *Lactobacillus reuteri* strain ATCC PTA 6475 demonstrated the ability to potently suppress human TNF production by LPS-activated monocytes and primary monocyte-derived macrophages from children with Crohn's disease (Belizário & Faintuch, 2018; Henke et al., 2019; C.-H. Lin et al., 2019; Tseng & Wu, 2019).

Importantly, the role of bioactive lipids in disease progression has been assessed: for instance, the resultant gastrointestinal dysbiosis observed in IBD patients, particularly impairs SCFA production. Such impairment presents consequences in energy supply to

colonocytes and local control of mucosal inflammation. As already mentioned, these patients show decreased butyrate-producing bacteria, especially *Faecalibacterium prausnitzii.* Nevertheless, alterations in other butyrate-producing species have been detected in ulcerative colitis patients: *Roseburia intestinalis* and *Roseburia hominis.* Regarding Crohn's disease patients studies have shown an increase of *Ruminococcus gnavus* and a decrease of *Faecalibacterium prausnitzii, Bifidobacterium adolescentis* and other SCFAs-producing bacteria (*Blautia faecis, Roseburia inulinivorans, Clostridium lavalense,* and *Bacteroides uniforms*) (Parada Venegas et al., 2019). These results indicate that the SCFA impairment may be related with disease onset and progression.

Moreover, regarding the endocannabinoid system, CB1 and CB2 receptors are recognized as having a protective role in IBD. It was observed that systemic oral administration of *Lactobacillus acidophilus* strain reduces abdominal pain through the involvement of cannabinoid receptors (Rousseaux et al., 2007) and it has been reported that PEA reduces inflammation in a mouse model of IBD (Alhouayek et al., 2015)**.** On the other hand, an increase in 2-AG endogenous levels protected against induced colitis in mice and reduces metabolic endotoxemia as well as the level of circulating inflammatory cytokines and peripheral brain inflammation. Furthermore, the authors suggested that increased 2-AG levels (Alhouayek et al., 2011), observed in *Akkermansia muciniphila* treatment may also contribute to reduced inflammation. Considering such results, targeting CB1 and CB2 receptors has been explored as a therapeutical approach.

PUFAs, like omega-3 PUFAs - EPA, DHA and/or ALA- are important regulators of inflammatory processes and some studies have been addressing their potential association with or beneficial effect in IBD. Several studies showed inconsistent results on the association between fish consumption and omega-3 PUFAs and the risk of IBD. Nevertheless, recently a meta-analysis study showed an inverse association between fish consumption and the risk of Crohn's disease as well as an inverse association between dietary long-chain omega-3 PUFAs intake and ulcerative colitis incidence, but further studies are required (Mozaffari et al., 2020). In addition, as far as we know, there are no studies specifically addressing the impact of PUFAs on microbiota and their positive role on IBD.

3.2. Obesity

Food additives, such as sweeteners and emulsifiers, found in processed foods have been shown to affect gut homeostasis and thus, the gut microbiota. Such additives have been connected to metabolic syndrome and chronic inflammatory diseases (Cani et al., 2016), suggesting a direct link between diet, namely western-pattern diet, obesity and microbiota dysregulation.

Obesity has been associated, in both human and animal studies, with low levels of Bacteroidetes and significantly overgrowth of bacteria from the Acidaminococcaceae family (Russo et al., 2017). Moreover, the intestinal microbiota and the endocannabinoid system have been suggested to interact during the development of obesity. Studies focusing on obesity have shown that on one hand modulation of the endocannabinoid system is associated with changes in gut microbiota, on the other hand, modulation of gut microbiota using probiotics, antibiotics, or germ-free mice,

affects endocannabinoid system signaling (Bisogno et al., 2021). Indeed, CB1 overactivity is considered a key contributor to the development of obesity. In fact, when a mouse model of diet-induced obesity is treated with a CB1 antagonist, microbiota changes are observed: *Akkermansia muciniphila* (Verrucomicrobia phylum), a mucin-degrading bacterium that resides in the mucus layer, increases and Lachnospiraceae and Erysipelotrichaceae levels decrease. Although Lachnospiraceae are SCFAs producers, different taxa of Lachnospiraceae are also associated with different intra- and extraintestinal diseases (Vacca et al., 2020). Regarding Erysipelotrichaceae, members of this bacterial family are described as highly immunogenic and are positively correlated with TNF levels (Kaakoush, 2015). Interestingly, *Akkermansia muciniphila* increases its abundance after OEA supplementation. Moreover, *Akkermansia muciniphila* supplementation is able to prevent the reduction of 2-PG levels in human patients and increased production of 2-PG, 2-OG and 2-AG in obese mice, suggesting an important connection between these important bacteria and the endocannabinoid system. On the other hand, mice treated with antibiotics selectively upregulated the expression of CB2 and exhibited altered microbiota profile as luminal counts of *Lactobacillus* and Enterobacteria were increased, whereas the *Clostridium* and the Verrucobacteria groups were reduced (as reviewed by (Bisogno et al., 2021)). Recently, high-fat, high-sucrose diet, induced microbiome disturbances as well as modifications of intestinal and circulating endocannabinoidome mediators, ultimately resulting in glucose intolerance, obesity and hyperinsulinemia in mice models. Moreover, the authors identified associations of specific bacterial genera - *Barnesiella, Adlercreutzia, Parasutterella, Propionibacterium, Enterococcus and Methylobacterium* - in the small intestine and cecum with increasing local and circulating levels of endocannabinoidome mediators, AEA (Lacroix Sébastien et al. 2019). Similar to described before, administration of *Akkermansia muciniphila* to HFD-fed mice led to an increase in intestinal levels of 2-AG, 2-OG and 2-PG, along with improved gut barrier function and decreased metabolic endotoxemia - a systemic, low-level elevation of gut-derived endotoxin (LPS). In addition, the administration of *Akkermansia muciniphila* limits the fat mass gained in HFD-fed mice and improves adipose tissue metabolism (Cani et al., 2016; Everard et al., 2013).

For instance, *Akkermansia muciniphila* can produce acetate and propionate (SCFAs) during this degradation process. Importantly, SCFAs may play a role in T-cell differentiation towards a more anti-inflammatory response. Indeed, these SCFAs are inversely associated with diet-induced obesity, adipose tissue inflammation and insulin resistance (Melbye et al., 2019). In addition, the role of SCFAs as signaling molecules show promising effects on obesity management: they are able to activate GPR41 and GPR43 thus, promoting leptin secretion by adipocytes, peptide YY and glucagon-like peptide 1 by enteroendocrine cells important to regulate host satiety (Tseng & Wu, 2019).

Although there is no clear evidence of a specific connection system between gut microbial-host signals and the onset or the progression of metabolic alterations associated with HFD, some studies have shown that intestinal epithelial MyD88 is a primary sensor involved in the cross-talk between nutrients, gut microbes and host during diet-induced obesity. The intestinal epithelial cell-specific deletion of MyD88 partially protects against diet-induced fat storage, inflammation and diabetes via mechanisms directly involving the gut microbiota. Importantly, AEA was decreased in the knockout mice whereas 2-AG and 2-OG were increased specifically during HFD

feeding. Importantly, 2-OG binds to the GPR119 receptor, stimulating the release of gut peptides such as glucagon-like peptide-1 and glucagon-like peptide-2 involved in glucose homeostasis and gut barrier function, respectively. Such results suggest that the improved glucose homeostasis, the reduced metabolic endotoxemia and the low-grade inflammatory state observed in the knockout mice are associated with the regulation of the intestinal endocannabinoid system. Moreover, the MyD88 deletion induced changes in the gut microbial community, as expected. The authors stated that among the differences the genus *Allobaculum* was decreased in wild-type (WT) HFD mice compared with WT control mice but was significantly increased in the MyD88 knockout HFD mice compared with the WT ones. Moreover, the authors demonstrated that transplanting gut microbes from the MyD88-knockout mice into germ-free mice replicated the protection against diet-induced metabolic diseases (Everard et al., 2014). MyD88 can be a mediator of endocannabinoid system interaction with gut microbiota in obesity models.

Interestingly, Robertson and collaborators recently show that murine maternal endogenous omega-3 PUFA production during gestation or lactation significantly reduces weight gain and markers of metabolic disruption in male offspring fed an HFD. Such effects appeared to be mediated by the restructuring of gut microbiota composition. Reduced maternal n-3 PUFA exposure led to significantly depleted *Epsilonproteobacteria*, *Bacteroides*, and *Akkermansia* and higher relative abundance of *Clostridia* (Robertson et al., 2018). In fact, the beneficial role of omega-3 fatty acids in obesity treatment is well documented, although such effect is thought to be mediated by a direct anti-inflammatory effect, an indirect effect on microbiota modulation has to be considered, but further studies are required.

3.3. Gut-brain axis: cognitive disorders

The CNS and the gastrointestinal tract are known to be in constant communication through a bidirectional pathway, recognized as the gut-brain axis. The gut-brain axis includes the CNS (brain and spinal cord), the autonomic and the ENS and peripheral nervous establishing an interdependency relationship between host-microbe and environment. This interaction influences both brain function and other distant organs since it is able to modulate the host's immune response and host cell proliferation and vascularization by regulating endocrine functions and neurological signaling (Baptista et al., 2020; Bisogno et al., 2021). It has been suggested that there is a direct interaction between gut microbiota and the ENS. Indeed, the gut microbiome is able to impact brain functions by influencing both host metabolism and through biological active mediators synthesis which ultimately reduce gut and BBB permeability, block microglia and astrocytes activation (inflammation mediators in CNS) triggering gut and brain homeostasis. They can also communicate by the production of neurotransmitters and SCFAs. Unfortunately, despite promising results suggesting a cross-talk between the gut microbiome and endocannabinoid system, the connection of the gut-brain axis and the endocannabinoid system in neurodegenerative diseases has never been deeply investigated (Bisogno et al., 2021).

Omega-3 and omega-6 PUFAs, particularly AA and DHA, are importantly present in CNS and are crucial for infant brain development. They are widely recognized to improve neurological outcomes and as discussed, they have demonstrated a beneficial impact on the gut microbiota ameliorating inflammatory responses. Recently, it has

been suggested that the neuroprotective effect of omega-3 is not only mediated by a direct incorporation into neural tissues but indirectly through their beneficial effects on gut microbiota (Robertson et al., 2017). Nonetheless, further investigations are required regarding this theme.

3.3.1. Multiple Sclerosis

MS is characterized as an autoimmune disease of the CNS, involving inflammatory processes at the BBB with the involvement, among other factors, of T cells. This inflammatory process leads to axonal damage and demyelination. In fact, CNS-specific T cells are normal components of the immune system when in a resting state, the problem is that when activated they migrate through secondary immune organs before they can cross BBB. When reaching the CNS, these T cells get re-activated and recruit other inflammatory cells leading to the mentioned demyelination process. This activation is shown to occur in the intestine (Haase et al., 2018). Interestingly, in relapsing-remitting multiple sclerosis (RRMS) study it was found that gut microbiota was altered, suggesting a role of the intestinal bacterial population in disease's progression. Specifically, *Methanobrevibacter* and *Akkermansia* bacteria genus were found in higher amounts in patients with RRMS, as well as *Blautia*, *Dorea* and *Pseudomonas*, involved in proinflammatory responses. While butyrate-producing *Butyricimonas* and *Faecalibacterium*, *Prevotella*, and *Clostridium* species known to produce SCFAs and previously associated with anti-inflammatory effects, were found in lower proportions compared to control subjects (Melbye et al., 2019; Russo et al., 2017). Moreover, recently, butyrate (SCFA) and caproic acid (medium-chain fatty acid (MCFA)) were found to be altered in serum concentrations of MS patients, comparing with the ones from healthy controls. Indeed, in the MS the concentration of butyrate was reduced while that of caproic acid was increased. Once again, the microbiota of these patients was depleted of butyrate-producing bacteria while it was enriched in mucin-degrading-proinflammatory components. The alterations observed in the SCFA and MCFAs concentrations were correlated with alterations of barrier permeability and inflammation. Thus, gut microbiota dysbiosis in MS patients is not only associated with altered butyrate concentration but instead with the SCFA/MCFA ratio (Saresella et al., 2020). Conflicting results have been reported regarding butyrate action: in a very recent study, the ratios of acetate/butyrate and acetate/(propionate + butyrate) were significantly lower in MS patients in a multivariate analysis. In this study, while the mentioned ratios and acetate levels correlated negatively with the proinflammatory biomarker IFNG, indicating an inverse relation between acetate and inflammation; butyrate concentration was found higher in MS patients, correlating positively with proinflammatory cytokines (IFNG and TNF) (Olsson et al., 2021). In fecal samples from RRMS patients, it was observed a lower abundance of *Parabacteroides distasonis*, which has been shown to increase T-cell differentiation into T-regulatory cells (Melbye et al., 2019). As mentioned, gut microbiota could be associated with the differentiation and regulation of Th17 cells in RRMS. Indeed, SCFAs are known to inhibit Th17 differentiation, and it is possible that for instance, *Prevotella* leads to higher levels of SCFAs than other SCFAs-producing bacteria. Indeed, Bacteroidetes is associated with a decreased Th1 differentiation and was decreased in MS patients in several studies. Interestingly, in mice colonized with fecal samples from MS subjects the IL-10 (anti-inflammatory cytokine) levels were lower when compared with mice colonized with fecal samples from healthy subjects (Melbye et al., 2019). A different study found that besides butyrate, the frequencies of circulating T-regulatory cells and T-helper cells

were positively correlated with serum levels of propionate, while acetate levels negatively correlate with TNF production (Trend et al., 2021). Regarding, acetate once again contradictory results were reported as high levels of this SCFA were found in MS patients when compared to healthy subjects. As the authors stated, considering such contradictory results, it is highly relevant to consider inflammatory background when exploring the association of microbiota metabolites and the immune system, and those basal patient conditions may change the influence of microbiota in the immune cells (Pérez-Pérez et al., 2020).

The activation of the endocannabinoid system has been shown to reduce neuroinflammation in experimental models of MS. Such effects are attributed to endocannabinoid's immunomodulatory and anti-inflammatory properties, targeting different cells like microglia and macrophages, cerebral endothelial cells, peripheral and central T lymphocytes, astrocytes. Besides, some studies have pointed out endocannabinoids' (specifically 2-AG) ability to promote remyelination by the enhancement of oligodendrocyte precursor cells through CB1 and CB2 receptors (as reviewed by (Mestre et al., 2018)). Nevertheless, studies in humans are scarce and not conclusive, being required more research in the field.

3.3.2. Alzheimer's disease

AD is characterized as a chronic age-related progressive neurodegenerative disorder that progresses from mild cognitive impairment to severe dementia over time (Bisogno et al., 2021). Gram-positive facultative anaerobic or microaerophilic *Lactobacillus* and other *Bifidobacterium* are capable of metabolizing glutamate to produce GABA, highly relevant since it is the major inhibitory neurotransmitter in the CNS. Importantly, the interaction between microbiota and N-methyl-D-aspartate glutamate receptor has been found, this receptor regulates synaptic plasticity and cognition. Moreover, OEA and PEA present a protective role in neuroinflammation oxidative stress and neurodegeneration (Russo et al., 2017). In the last years, endocannabinoid system modulation has been suggested and studied as a potential therapeutical approach for the treatment of AD. Indeed, human studies showed that CB2 receptors are overexpressed in neuritic plaque-associated glia analyzed in brains from AD patients. Furthermore, the expression of CB2 is related to amyloid-beta deposition - a characteristic feature of AD patients - suggesting a possible regulatory role associated with the pathological alterations of AD induced in microglial cells (Bisogno et al., 2021). Nevertheless, further studies are required to clarify such a role.

Similar to the remaining disorders discussed in this chapter, AD patients present an altered gut microbiome characterized by reduced species diversity and increased abundance of Bacteroidetes. Indeed, in AD mice models, microbiota composition and diversity were shown to be altered and the level of SCFAs was reduced, with alterations in metabolic pathways that are thought to be associated with amyloid deposition (Zhang et al., 2017). Nevertheless, other studies suggested that SCFAs interfere with protein-protein interactions between amyloid-beta peptides, disrupting their assembly into neurotoxic oligomers (Ho et al., 2018). Interestingly, through a germ-free amyloidosis mouse model SCFA were identified as the key mediator contributing to amyloid-beta plaque deposition, being microglia the key cell population responsive to SCFA (Colombo et al., 2021). However, these results seem contradictory when comparing to what was already discussed and considering the possible beneficial role of SCFA on

microbiota modulation. In fact, SCFA in neurodegenerative conditions has been associated with microglia reactivity and activation. On the other hand, their function in primary autoimmune and acute brain disorders has mainly been described to be anti-inflammatory. The detailed reasons for such divergent functions in different disorders are currently unknown.

3.3.3. Parkinson's disease

PD is a chronic, progressive, multisystem neurodegenerative movement disorder resulting in characteristic motor symptoms including resting tremor, bradykinesia, rigidity, and gait abnormalities, as well as non-motor symptoms such as hyposmia, sleep disorders, depression, and gastrointestinal symptoms (Shen et al., 2021). Most PD patients present gastrointestinal manifestations known to be caused by ENS disturbances, thus the hypothesis of a relationship between the gut microbiota and the development of this disease has been gaining interest. In a similar way as described for MS, PD patients present a microbiota related with an increased inflammatory phenotype, where SCFA concentration was found to be decreased (Aho et al., 2021); indeed, PD subjects presented lower concentrations of bacteria from *Blantia*, *Coprococcus* and *Roseburia* genera but increased concentrations of *Ralstonia* proteobacteria genus and Enterobacteriacea and Prevotellaceae (Russo et al., 2017).

Aggregation of the protein α-synuclein (αSyn) is described as the main pathogenic event of PD, affecting dopaminergic neurons. Indeed, using a mouse model of αSyn overexpression, it was observed that gut microbiota is required to elicit pathophysiological alterations since the elimination of the gut microbiota with antibiotics ameliorates the condition (Sampson et al., 2016; Silva et al., 2020). Moreover, supporting these results, fecal microbiota transplantation from healthy donors and butyrate administration in PD animal models, improves both motor impairment and dopamine deficiency (Liu et al., 2017; Sharma et al., 2015; St. Laurent et al., 2013; Sun et al., 2018). These results suggest that SCFA levels related to microglia alteration are implicated in PD onset and progression. Nevertheless, the role of SCFAs on PD continues to be controversial.

4. Conclusion

Interestingly, bioactive lipids exert both pro- and anti-inflammatory actions on gut microbiota, influencing immune regulation, inflammation and homeostasis. The endocannabinoid system, specifically, is involved in modulation of gastric emptying, gastrointestinal motility and inflammation. On the other hand, SCFAs are able to maintain epithelial membrane integrity by regulating both mucus production and tight junction expression. In addition, SCFAs are recognized to interact with BBB and modulate brain function. SCFAs are able to modulate T-cell differentiation towards regulatory subtypes and suppress proinflammatory responses (TH17 and Th1 cells), having a role on inflammatory processes. Lastly, omega-3's potential action in gut microbiota lies in restoring the dysbiosis that is encountered in several pathologies, functioning as prebiotics. The positive effects of omega-3 fatty acids in gut microbiota and associated pathologies are closely connected to their anti-inflammatory capacity, nonetheless and despite promising results, there is still a lack of knowledge regarding this theme and further studies are warranted (Figure 1).

The role of these bioactive lipids in gut microbiota was described for different pathologies: in IBD the resultant gastrointestinal dysbiosis observed, particularly impairs SCFA production. Moreover, in IBD targeting CB1 and CB2 receptors (receptors from the endocannabinoid system) has been explored as a therapeutical approach. Moreover, in inflammatory diseases, such as IBD and obesity, SCFAs develop an important role since they can also act as signaling molecules to activate GPR receptors. Endocannabinoid system has been suggested as a possible link between microbiota and adipose tissue, developing an important role in obesity. Additionally, the gut microbiome is able to impact brain functions by influencing both host metabolism and through biological active mediators' synthesis which ultimately reduce gut and BBB permeability, block microglia and astrocytes activation triggering gut and brain homeostasis. They can also communicate by the production of neurotransmitters and SCFAs. Unfortunately, despite promising results suggesting a cross-talk between the gut microbiome and endocannabinoid system, the connection of the gut-brain axis and the endocannabinoid system in neurodegenerative diseases, like MS, AD and PD, has never been deeply investigated and the role of SCFAs is still controverse.

5. List of Figures

Figure 1 – Gut microbiota importantly maintains host's homeostasis.

6. References

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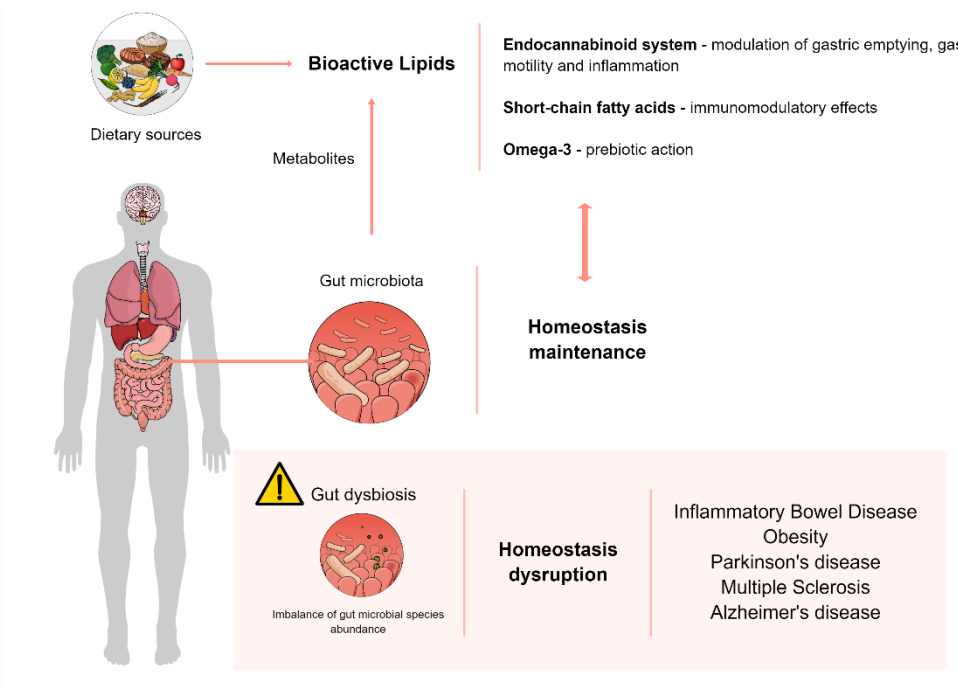

Figure 2 – Gut microbiota importantly maintains host's homeostasis. When there is imbalance of gut microbial species richness, defined as gut dysbiosis, there is the development of several pathologies like inflammatory bowel disease, obesity, and central nervous system disorders – Parkinson's disease, Alzheimer's disease and Multiple Sclerosis. The gut microbiota homeostasis is closely connected with bioactive lipids action. Indeed, the endocannabinoid system maintains gut physiology through modulation of gastric emptying, gastrointestinal motility and inflammation. Short-chain fatty acids are metabolites produced by bacterial fermentation of non-digestible carbohydrates such as dietary fibers and resistant starch, that present immunomodulatory effects. Several of the mentioned diseases are associated with impairment of short-chain fatty acids producing species. Omega-3 fatty acids present anti-inflammatory effects and help restoring dysbiosis, functioning as prebiotics.