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Department of Biomolecular Sciences

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GUT MICROBIOTA MODULATION BY DIET AND EXERCISE: EFFECTS IN THE PREVENTION OF NON-COMMUNICABLE DEGENERATIVE DISEASES AND IMPROVEMENT OF QUALITY OF LIFE

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Abstract

The gut microbiota (GM) is a central regulator of host homeostasis, influencing metabolic, immune, and neuroendocrine signaling. Dysbiosis has been implicated in the onset and progression of neurodegenerative and oncological diseases. This doctoral research examined microbiota–host interactions across three complementary clinical and experimental models.

In the first study, GM composition was characterized in 109 patients with Parkinson's disease (PD), clinically stratified by disease severity and presence of motor fluctuations. Disease progression was associated with a depletion of butyrate-producing taxa (e.g., *Faecalibacterium*, *Roseburia*) and an enrichment of mucolytic and pro-inflammatory genera (e.g., *Alistipes*, *Collinsella*, *Desulfovibrio*). Patients experiencing motor fluctuations exhibited a distinct dysbiotic profile, suggesting that microbial metabolic imbalance may contribute to impaired intestinal barrier function, systemic inflammation, and dopaminergic instability along the gut–brain axis.

The second project investigated a 12-week home-based Mediterranean diet and aerobic exercise intervention in breast cancer survivors (n = 20). In addition to improvements in cardiometabolic biomarkers, GM analysis demonstrated a reduction in Proteobacteria—often linked to inflammation—and positive associations between Mediterranean diet adherence and butyrate-producing genera. These findings indicate that lifestyle modification can promote a microbiota configuration associated with improved metabolic and inflammatory status during survivorship.

The third study explored microbiota-derived metabolites as modulators of tumor cell plasticity in non-small cell lung cancer (NSCLC). Propionate-rich supernatants from *Acidopropionibacterium microaerophilum* decreased ZEB1 and Vimentin expression while increasing E-cadherin in A549 cells, indicating inhibition of epithelial-to-mesenchymal transition (EMT). This supports a role for microbial metabolites in modulating tumor behavior through metabolic–epigenetic pathways.

Across neurological and oncological contexts, the GM emerges as both a biomarker of disease state and a tractable therapeutic target. Diet, exercise, and therapeutic strategies that reshape microbial composition or exploit microbial metabolite production may provide innovative avenues to mitigate neuroinflammation, enhance metabolic recovery after cancer therapy, and limit tumor progression.

Summary

1. Introduction

The human gut harbors one of the most densely populated microbial ecosystems on Earth, comprising over 100 trillion microorganisms and an estimated quadrillion viral particles [1–3]. Throughout evolution, humans have co-adapted with this vast microbial consortium, resulting in highly individualized ecosystems that continuously respond to host physiology, diet, and environment. This dynamic crosstalk sustains a finely balanced symbiosis, enabling both host and microbes to thrive within a constantly shifting biological landscape [4].

Beyond ecological coexistence, the gut microbiota (GM) performs essential functions, including nutrient metabolism and fermentation, as well as immune regulation and pathogen defense. Perturbations of this balance—commonly referred to as dysbiosis—have been implicated in the initiation and progression of a wide spectrum of diseases [5].

Functionally, the gut microbiome operates as a highly dynamic yet resilient network, in which the collective repertoire of microbial activities exerts a profound influence on host immunity and systemic physiology [6]. Within this framework, the notion of a “functional core” has emerged: a conserved set of metabolic and molecular pathways that remain stable across individuals, even when taxonomic composition diverges. Thus, two individuals may harbor distinct microbial species, yet their communities may converge functionally through analogous biochemical processes such as short-chain fatty acid (SCFA) production, bile acid biotransformation, and modulation of host immune signaling. This functional redundancy underlines the ecological resilience of the microbiome, ensuring preservation of host-supportive functions despite taxonomic variability [3].

A growing body of evidence highlights how dysbiosis contributes to disease pathogenesis not only within the gastrointestinal tract (GIT), but also in extraintestinal tissues and organ systems[7]. Central to these effects is the complex bidirectional crosstalk between microbial communities and host eukaryotic cells, which influences immune responses, metabolic pathways, and susceptibility to inflammatory or metabolic disorders. Disruptions in this communication have been associated, for example, with inflammatory bowel disease (IBD), obesity, insulin resistance, and cardiovascular dysfunction. Understanding these mechanisms may provide a promising avenue for developing innovative microbiota-based strategies for prevention and therapy [8].

The central objective of the investigation is to analyze the composition of GM across different health conditions, comparing microbial signatures among groups and identifying distinct bacterial community clusters. Additionally, this study aims to investigate the functional implications of

microbe–host interactions, with the goal of elucidating how variations in microbial community structure may contribute to differential disease susceptibility and physiological outcomes.

2. Gut microbiota

The human GM is a complex and dynamic ecosystem inhabited by a wide variety of microorganisms, including bacteria, fungi, archaea, and viruses. These microorganisms interact both with each other and with the human host, beginning to colonize the gut at birth and continuing to evolve alongside the host throughout their lifespan [8,9]. The gut microbiome contributes the majority of the genetic information in our metagenome, influencing our resistance and susceptibility to diseases, prevalent inflammatory conditions such as type 1 diabetes, ulcerative colitis, and Crohn's disease [10].

The human body, when exposed to the external environment, becomes colonized by microbes. Among the different body sites colonized by microorganisms, such as mouth, vagina, skin, and lungs, the gastrointestinal tract represents the most densely populated niche, with the large intestine harboring the most significant microbial diversity. Most human microbiome research focuses on the microbial community of the large intestine, which harbors the greatest microbial diversity and is commonly studied through the analysis of stool samples [11]. Consequently, this has made the gut microbiome the central focus of most human microbiome research, which increasingly highlights its pivotal role in host metabolism, nutrition, immune regulation, and disease susceptibility [3].

Large-scale projects such as Human Microbiome Project [12,13] and MetaHIT [14] have characterized the GM, revealing substantial variability among individuals. This was made possible by advancements in next-generation sequencing technologies.

GM are composed of several species of microorganisms, including bacteria, yeast, and viruses. From a taxonomic perspective, bacterial taxa are classified according to phyla, classes, orders, families, genera, species, and strains. Despite this diversity, only a limited number of bacterial phyla predominates, encompassing more than 160 identified species [15].

Studies have shown that approximately 90% of the bacterial phyla found in the human gut belong to Bacteroidetes and Firmicutes, while Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia represent the remaining minority (**Figure 1**).

Figure 1. Examples of taxonomic gut microbiota composition

In the box are cited examples of bacteria belonging to Phyla Firmicutes and Bacteroidetes, representing 90% of gut microbiota [16].

The phylum Firmicutes consists largely of gram-positive bacteria, obligately anaerobic species, including *Clostridium* (95%), *Lactobacillus*, *Bacillus*, *Enterococcus*, and *Ruminococcus*, while Bacteroidetes are gram-negative bacteria predominantly composed of *Bacteroides* and *Prevotella*. Actinobacteria are represented primarily by *Bifidobacterium*, while Proteobacteria include gram-negative facultative anaerobes such as *Escherichia* and *Klebsiella* [9].

A summary of the main phyla and representative genera is provided in **Table 1**.

In addition to shifts in the relative abundances of key bacterial taxa, species diversity is a critical indicator of GM health and homeostasis. Healthy individuals harbor thousands of bacterial species, reflecting the high richness of a stable microbial ecosystem. Diversity is commonly quantified using indices such as Shannon Index [17]. Reductions in species richness or marked shifts in dominant phyla are frequently associated with pathological state, including obesity and IBD [18].

Phylum (20221 revised nomenclature)	**Brief description**	**Genus examples**
Bacteroidetes (Bacteroidota)	Gram-negative; typically obligately anaerobic; often abundant	*Bacteroides, Prevotella*
Firmicutes (Bacillota)	Gram-positive; typically obligately anaerobic; often abundant; highly diverse phyla	*Clostridium, Enterococcus, Eubacterium, Faecalibacterium, Lactobacillus, Roseburia, Ruminococcus, Streptococcus*
Actinobacteria (Actinomycetota)	Gram-positive; typically obligately anaerobic/requires low oxygen levels	*Bifidobacterium, Corynebacterium, Eggerthella*
Proteobacteria (Pseudomonadota)	Gram-negative; mostly facultatively anaerobic; includes many pathogenic species	*Enterobacter, Escherichia, Klebsiella, Serratia*

Table 1. Summary of the four predominant bacterial phyla in the gut microbiome, presented in order of their typical relative abundance in healthy individuals.

In addition to diversity-based measures of microbial complexity, another relevant framework for describing gut community structure is the concept of enterotypes, which classify individuals into three predominant clusters characterized by the dominance of *Bacteroides*, *Prevotella*, or *Ruminococcus* [19]. Subsequent analyses, however, have suggested that the *Ruminococcus* group

may not represent an independent cluster but rather overlap with *Bacteroides*-enriched communities [20]. Enterotypes are thought to be strongly influenced by long-term dietary habits, and although their precise functional significance is still under discussion, they provide a valuable tool for interpreting interindividual differences in GM composition [3].

Beyond bacteria, increasing attention has been directed toward other components of the GM, including eukaryotic microorganisms and viruses. Emerging evidence indicates that even healthy individuals host a complex viral community, collectively referred to as the human virome [21–24]. Current estimates likely underestimate virome diversity, as widely used extraction techniques preferentially detect encapsulated viral particles. Similar to bacterial communities, the virome displays high interindividual variability and may exert significant influence on host physiology and microbe–microbe interactions [25,26].

2.1 Structure and Function of the Intestinal Tract

The GIT is a continuous tubular system extending from the oral cavity to the anus, measuring approximately ten meters in length. While its primary role is the digestion and absorption of nutrients, the GIT also performs critical regulatory and immune functions, maintaining homeostasis with the resident microbiota (**Figure 2**).

The small intestine, comprising the duodenum, jejunum, and ileum, is specialized for nutrient uptake. The duodenojejunal flexure, supported by the ligament of Treitz, marks the transition from duodenum to jejunum, whereas the shift to the ileum is more gradual. Morphologically, the jejunum has a thicker wall, narrower lumen, and more prominent circular folds, while the distal ileum contains more mesenteric adipose tissue and Peyer's patches.

The large intestine, in contrast, lacks villi and consists primarily of deep crypts designed for water and electrolyte absorption rather than nutrient uptake. It can be divided into right and left segments, reflecting embryological origin, vascular supply, and innervation: the right colon derives from the midgut and includes the caecum, appendix, ascending, and proximal transverse colon, whereas the left colon originates from the hindgut and comprises the distal transverse colon, descending colon, sigmoid colon, and rectum.

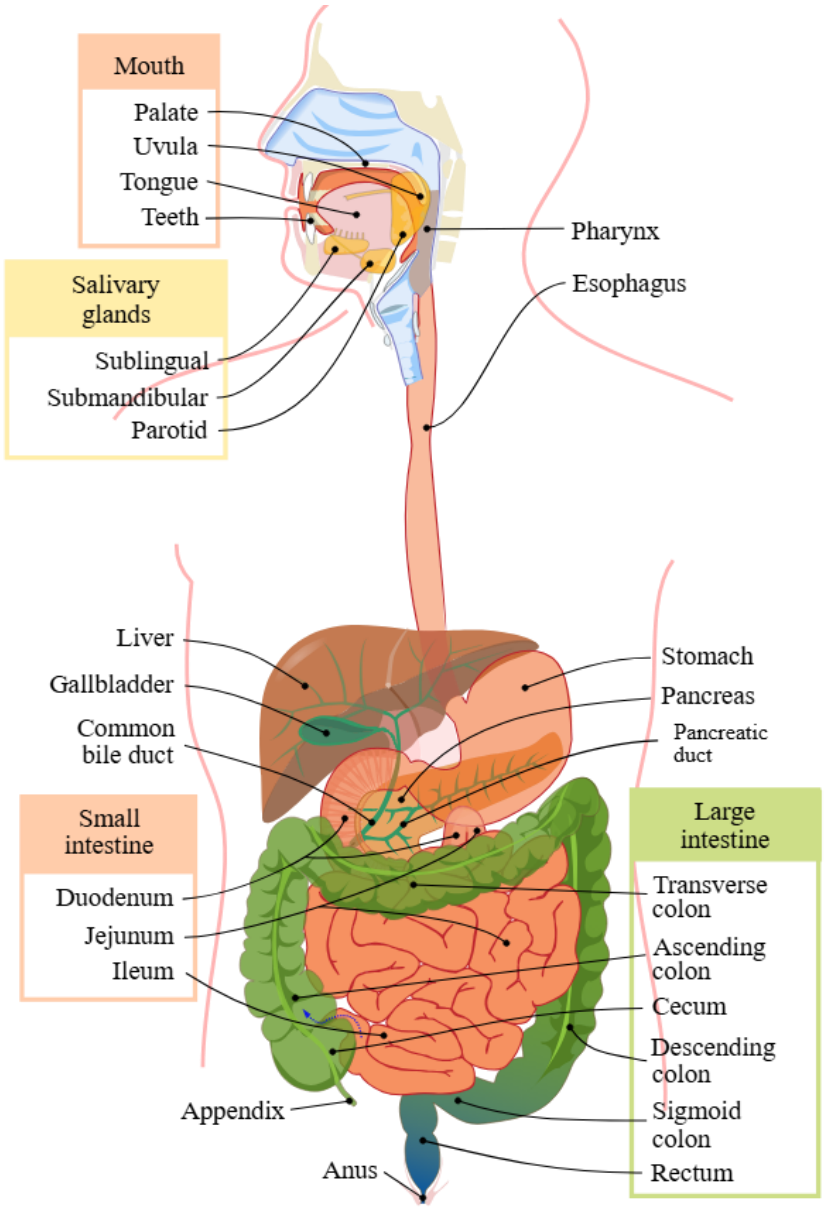

Figure 2. **Human gastrointestinal tract** [27]

Histologically, the intestinal wall consists of mucosa, submucosa, muscularis propria, and serosa or adventitia (**Figure 3**). The mucosa is composed of the epithelial layer, lamina propria, and muscularis mucosa—the latter serving as an important landmark in tumor staging.

The epithelial surface of the small intestine is arranged into villi projecting into the lumen and crypts of Lieberkühn extending downward into the lamina propria. Villi increase the absorptive surface area, whereas crypts contain the proliferative niche that continually renews the epithelium. This renewal is driven by Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5)-positive stem cells, regulated by Wnt/β-catenin and Notch signaling pathways, and supported by surrounding telocytes, immune factors, and microbial metabolites such as SCFAs.

In contrast, the large intestine lacks villi and is characterized by deep crypts with a higher proportion of goblet cells and progenitor cells, reflecting its primary roles in mucus production and water and electrolyte absorption [28].

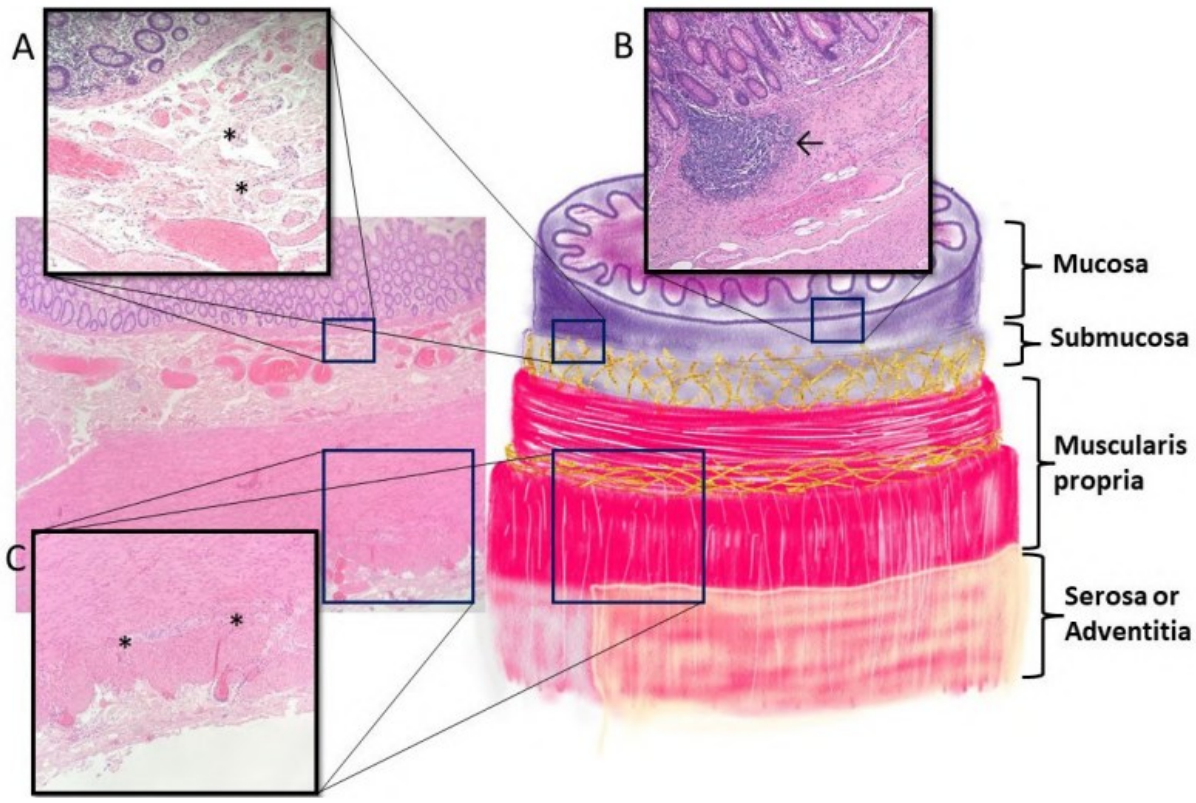

Figure 3. Histological and schematic representation of the intestinal wall

The image illustrates the four main layers of the intestinal wall: mucosa, submucosa, muscularis propria, and serosa/adventitia. **A)** Submucosa containing loose connective tissue and vascular structures. **B)** Gut-associated lymphoid tissue (GALT), consistent with a Peyer's patch. **C)** Muscularis propria composed of inner circular and outer longitudinal smooth muscle layers involved in peristalsis [29].

The intestinal epithelium consists of several specialized cell types [28]:

- Enterocytes, responsible for nutrient absorption and ion transport,
- Goblet cells, which secrete gel-forming mucins,
- Paneth cells, which release antimicrobial peptides within the crypt base,
- Enteroendocrine cells (EECs), which coordinate local and systemic signaling,
- Tuft cells, involved in chemosensing and type 2 immune signaling,

- Microfold (M) cells, which transport luminal antigens to Peyer's patches to initiate mucosal immune responses.

Overlaying the epithelium is the mucus layer, composed mainly of water and gel-forming mucins, most notably Mucin-2 (MUC2) in the intestine and Mucin-5AC (MUC5AC) in the stomach. These mucins are secreted by goblet cells, forming a single, loosely organized mucus layer in the small intestine and a dense two-layered system in the colon. The mucus acts as a physical and chemical barrier, protecting epithelial cells from toxins and pathogens while allowing nutrient absorption. The integrity of this barrier is modulated by the GM, immune signals, and diet [30].

Germ-free animals exhibit a reduced mucus layers and a decreased number of goblet cells, highlighting the importance of microbial signaling molecules such as lipopolysaccharide (LPS) and peptidoglycan in mucus regulation. Certain commensal bacteria, including *Akkermansia muciniphila* and *Bacteroides thetaiotaomicron*, can metabolize mucin glycans, contributing to mucus turnover and microbial homeostasis. Additionally, cytokines modulate mucus dynamics: interleukin-4 (IL-4) promotes goblet cell expansion and increases mucus thickness, whereas interleukin-18 (IL-18) reduces goblet cell numbers, demonstrating the role of immune regulation in shaping the mucus barrier.

Together, the epithelial cells, the mucus layer, and the intercellular junctional complexes constitute the intestinal barrier, a dynamic interface that separates the host from the external environment while allowing controlled nutrient exchange. Beneath the mucus layer, the epithelium forms a selective permeability barrier maintained by tight junctions, adherens junctions, desmosomes, and gap junctions. Tight junctions, composed of claudins, occludin, and tricellulin, regulate paracellular permeability, ensuring spatial segregation between luminal contents and the lamina propria while allowing selective transport of ions and small solutes [31].

The GM plays a central role in nutrient metabolism, barrier reinforcement, and immune regulation. Microbial fermentation of undigested carbohydrates produces SCFAs such as butyrate, acetate, and propionate, which serve as energy substrates for epithelial cells, promote tight junction assembly via AMP-activated protein kinase (AMPK) activation, and stimulate mucus production. Microbial signals, recognized as microbe-associated molecular patterns (MAMPs) by Toll-like receptors (TLRs) on epithelial and immune cells, activate Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB)-mediated pathways, inducing immune responses while maintaining tolerance to commensals.

Certain species, including Bacteroides *fragilis*, promote regulatory T-cell expansion and IL-10 secretion, contributing to anti-inflammatory homeostasis. Underlying the epithelium, the lamina propria contains innate and adaptive immune cells, including lymphocytes, macrophages, dendritic cells, and plasma cells producing immunoglobulin A (IgA). Peyer's patches serve as inductive sites for mucosal immunity, facilitating antigen sampling and IgA secretion. Interleukin-22 (IL-22), produced by both epithelial and immune cells, supports epithelial regeneration, mucus homeostasis, and antimicrobial peptide release (**Figure 4**). Dysregulation of these mechanisms is implicated in diseases such as Crohn's disease, ulcerative colitis, and increased susceptibility to infection [31].

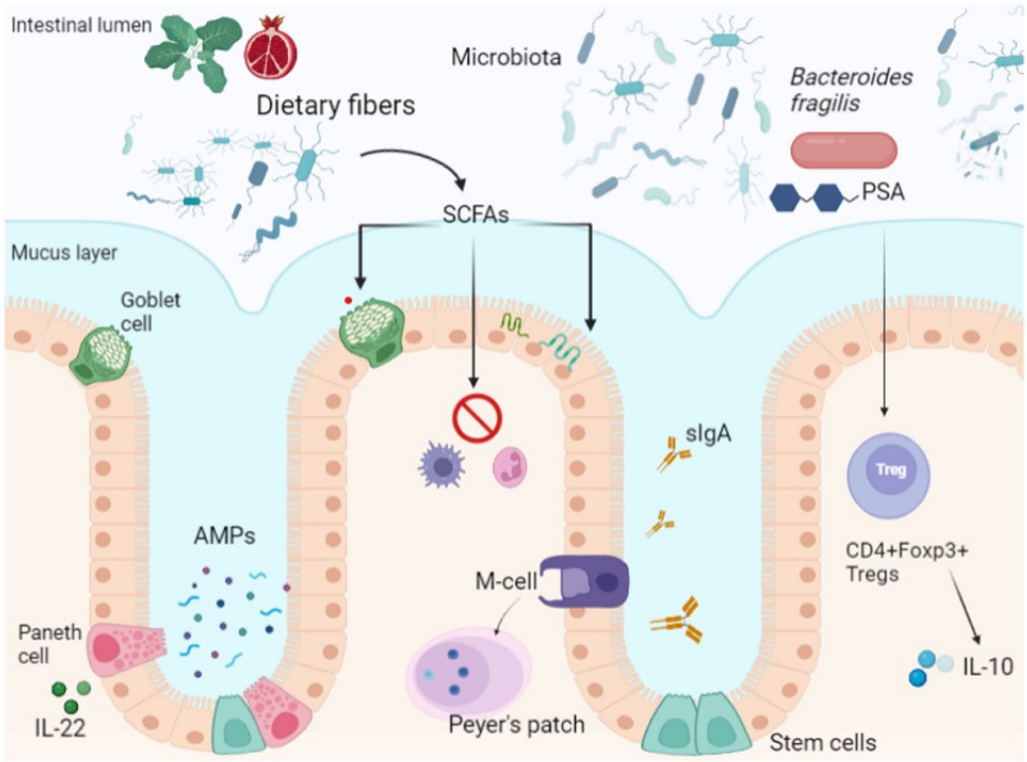

Figure 4. Intestinal barrier composition

SCFAs: Short chain fatty acids; M-Cells: Microfold cells; PSA: Polysaccharide A; AMPs: antimicrobial peptides; sIgA: secretory IgA [31].

2.2 Evolution of gut microbiota

In recent years, numerous studies have focused on human microbiota, which is now recognized as the collection of microorganisms residing in the gastrointestinal tract.

The human body is a symbiotic organism composed of both eukaryotic and prokaryotic cells, including the microbiota. The composition of the gut microbiome is specific to each host and evolves throughout an individual's life. It is influenced by exogenous and endogenous factors, such as pregnancy, childbirth, lactation, stress, diet, and physical activity [32].

Every newborn is born with a nearly germ-free environment, as the uterus provides a sterile atmosphere. However, recent studies have shown that bacteria can be present in amniotic fluid. This initial germ-free state lasts only for a short time after birth. When the baby passes through the vagina, they are exposed to maternal bacteria that begin to colonize the mucosal surfaces of the digestive, respiratory, and urogenital tracts, as well as the skin [33].

Children born vaginally have gastrointestinal microbiota dominated by Actinobacteria (*Bifidobacterium*, *Atobium*), Firmicutes (*Lactobacillus*, *Megamonas*), Bacteroidetes (*Prevotella*, *Bacteroides, Parabacteroides*), Fusobacteria (*Sneathia*), and Proteobacteria (*Shigella, Escherichia*), in contrast to those delivered via caesarean section (C-section). These bacteria produce SCFAs, which lower luminal pH and inhibit pathogen growth. Vaginally delivered children also tend to have higher microbial diversity and lower levels of *Clostridium difficile* in their microbiota [34–43].

In contrast, the intestinal microbiota following a cesarean section (C-section) is marked by delayed bacterial colonization and reduced diversity, along with an increased presence of opportunistic pathogens associated with the hospital environment. After a cesarean delivery, the gastrointestinal microbiome is primarily dominated by Firmicutes, including genera such as *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Clostridium*, and *Veillonella*, as well as Proteobacteria, which includes *Klebsiella*, *Enterobacter*, and *Haemophilus* [37,39,42,44–49].

These differences occur because bacteria from the mother's vagina and gut are transferred during vaginal delivery, whereas during a cesarean birth, bacteria from the mother's skin are transmitted [50].

The type of nutrition a baby receives during the first few months of life influences the composition of their microbiota. Studies suggest that individuals who were not breastfed may have a higher likelihood of developing food intolerances later in life. Studies show that the intestinal microbiota

of formula-fed infants contains more potential pathogens compared to breastfed children. This microbiota is primarily dominated by Firmicutes (such as *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Lactobacillus*, and *Clostridium*), Bacteroidetes (primarily *Bacteroides*), Proteobacteria (particularly *Enterobacteria*), and Actinobacteria (like *Atopobium*) [51–54].

Research indicates that children born vaginally at term without instrumental assistance and fed maternal breast milk have the highest likelihood of developing a healthy gastrointestinal microbiota, which helps prevent dysbiosis [55,56].

Throughout gestational age and continuing afterward, the gut microbiome undergoes colonization through contact with the external environment.

Despite significant individual variations, the development of the microbiota generally follows predictable patterns, as illustrated in **Figure 5**. Immediately after birth, healthy term infants possess an aerobic GIT that encourages the growth of facultative anaerobic bacteria, such as Firmicutes (*Enterococcus, Staphylococcus, Streptococcus*) and Proteobacteria (*Enterobacter*, *Escherichia* coli). These bacteria lower oxygen levels, which allows obligate anaerobes, including Actinobacteria (*Bifidobacterium*), Bacteroidetes (*Bacteroides*), and certain Firmicutes (*Clostridium, Lactobacillus, Ruminococcus*), to thrive [57–62].

By three months of age, the intestinal tract is predominantly occupied by Actinobacteria (*Bifidobacterium*), Bacteroidetes (*Bacteroides*), and Proteobacteria (*Escherichia*). At 12 months, the GIT primarily consists of Actinobacteria (*Bifidobacterium, Collinsella*) and Firmicutes (*Lactobacillus, Megasphaera, Veillonella*). Between the ages of 2 and 3, children exhibit greater diversity in their microbiota with reduced individual differences. After approximately 3 years, the microbiota stabilizes and begins to resemble adult microbiota in terms of diversity and complexity, with Firmicutes and Bacteroidetes being the dominant groups [19,45,53,63,64].

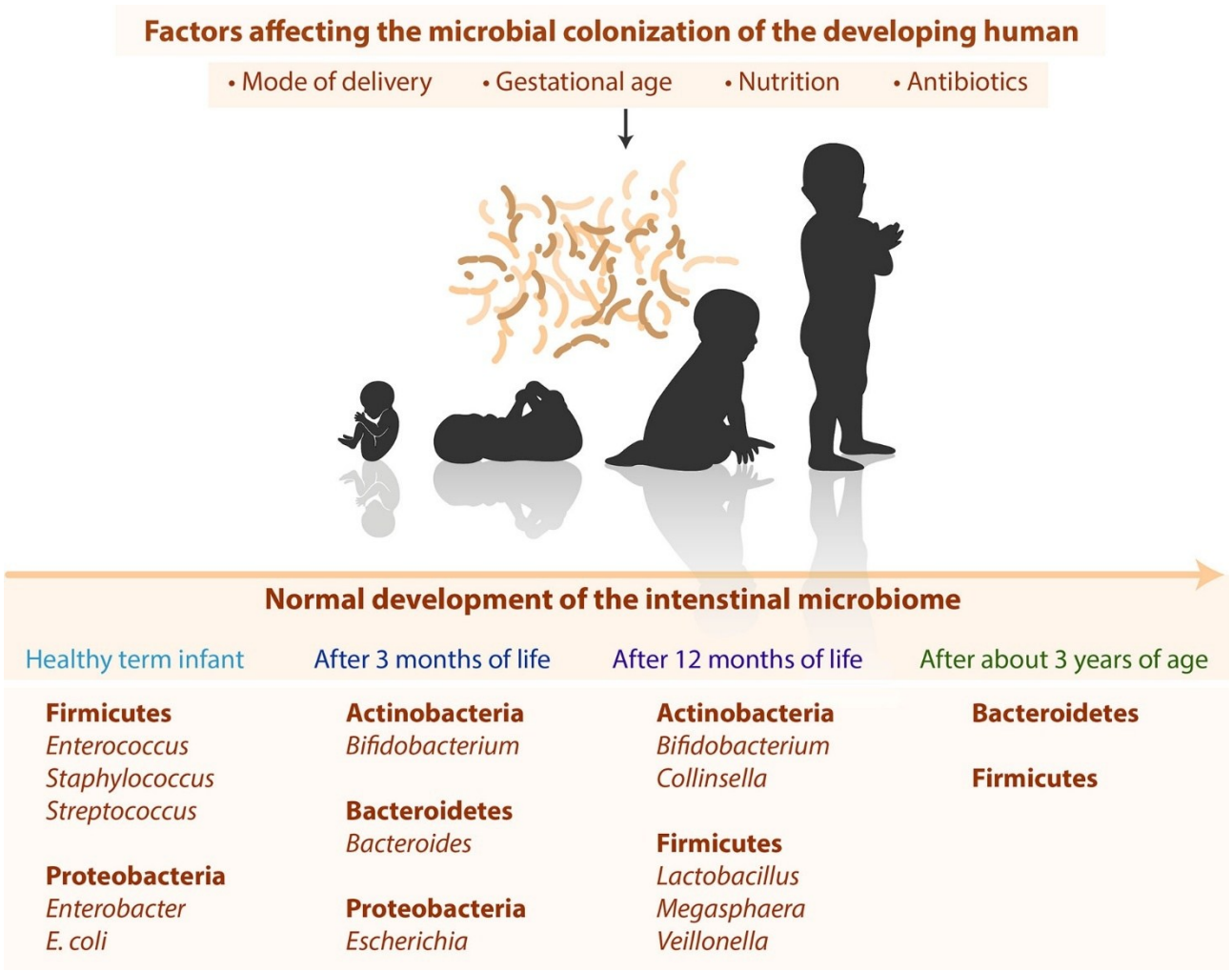

Figure 5. The normal development of a child's GM during gestation and early growth stages and the principal factors affecting the microbial colonization [50]

By the end of this process and throughout life, each individual becomes host to approximately one hundred trillion microbes, outnumbering the total number of cells in the body. There are over a thousand different microbial species present, and their collective metagenome contains around one hundred times more genes than the human genome [33]. In adulthood, the composition of gut bacteria generally remains stable until old age. However, in elderly individuals, the diversity of microbiota tends to increase. This change may be related to the broader range of health issues associated with aging and the various treatments that arise from those conditions [3].

2.3 How is a healthy microbiome defined?

The composition and structure of the human microbiome have been extensively studied through various microbiome projects linked to a global network. These include the International Human Microbiome Consortium, the European Commission's Metagenomics of the Human Intestinal Tract project, the US National Institutes of Health's Human Microbiome Project, and the Canadian Microbiome Initiative, among others. The objective is to define a healthy microbiota and identify the primary factors that influence it, affecting human health [65].

A healthy microbiome is generally characterized by high microbial diversity, functional redundancy, metabolic balance, and the prevalence of beneficial taxa involved in nutrient processing, immune modulation, pathogen exclusion, anti-inflammatory signaling, and SCFA production [65]. Although there is no single universal "healthy" microbial profile due to substantial interindividual variability, these core functional attributes are consistently observed.

The diverse composition of microbes, along with their various metabolic activities, plays a significant role in shaping the host's immune response. Genetic predisposition and environmental factors—particularly diet—further modulate how the host interacts with the microbiota, creating a dynamic and individualized host–microbe relationship that influences both health status and therapeutic outcomes [65].

The human microbiome also exhibits marked spatial specialization. Samples from different body sites (skin, oral cavity, gut, and vagina) reveal that while no single microbial taxon is present across all niches, metabolic pathways show remarkable conservation, underscoring the concept of functional redundancy (**Figure 6**) [66].

An additional hallmark of a healthy microbiota is its ecological stability, described in terms of resistance and resilience. Resistance denotes the ability of the microbial community to endure ecological stress, while resilience reflects its capacity to recover and return to equilibrium once the stress has subsided. A lack of diversity or an overly uniform community structure may weaken these properties, making the microbiota more vulnerable to disturbances and, ultimately, less stable [10,65].

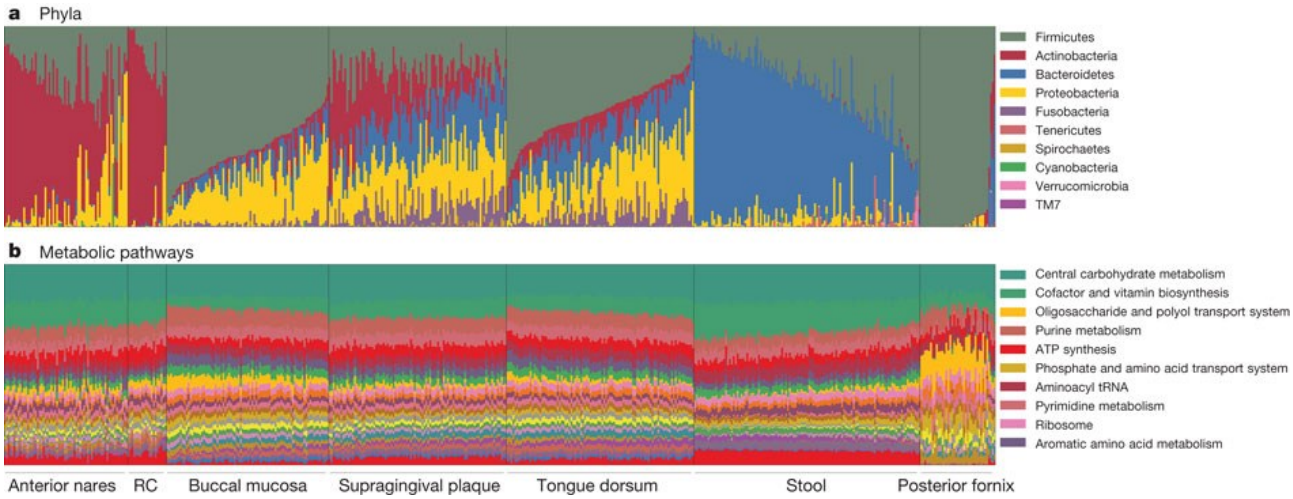

Figure 6. Carriage of microbial taxa varies, while metabolic pathways remain stable within a healthy population [66]

2.4 Role of a balanced gut microbiota

A healthy gut microbiome contributes to multiple aspects of host physiology, including digestion, nutrient absorption, and energy metabolism. It supports immune system development and regulation, protects against pathogenic microbes, reinforces the integrity of the intestinal barrier, and modulates the production of microbial metabolites that influence both local and systemic processes (**Figure 7**). The following sections detail key functional roles of the GM and their relevance to host physiology.

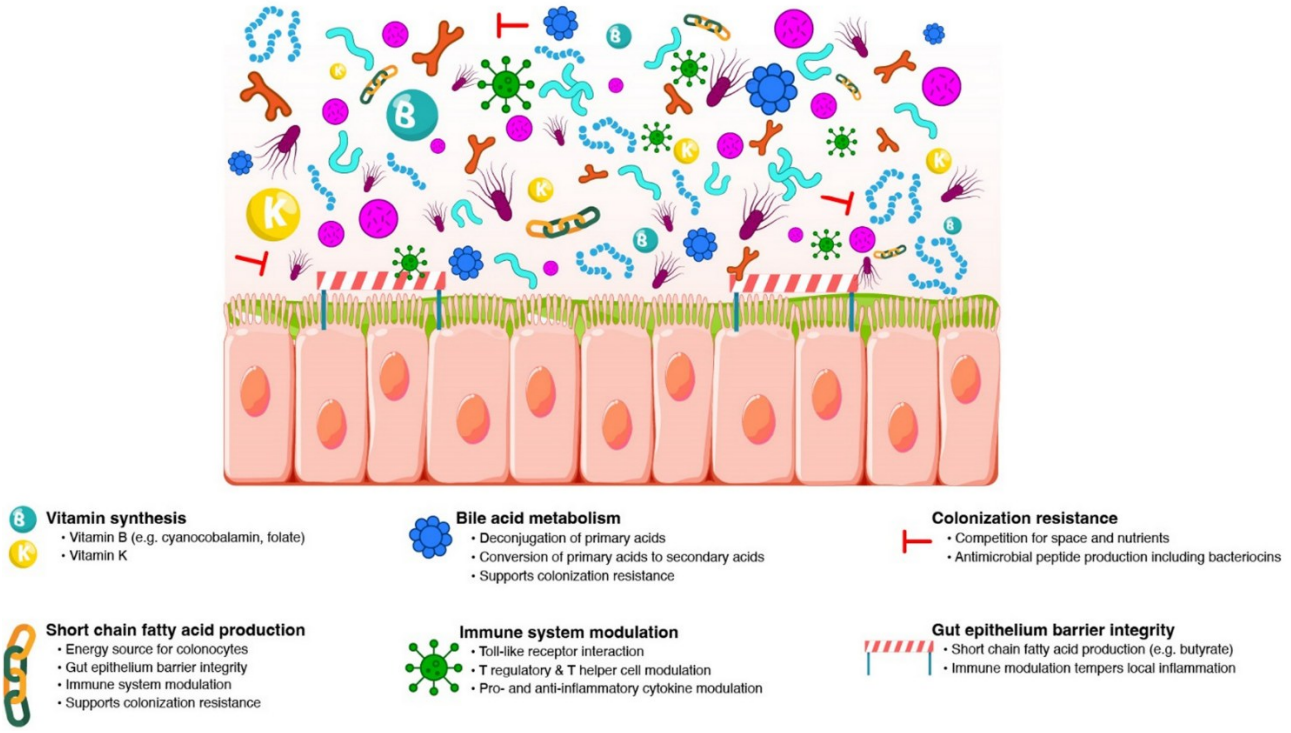

Figure 7. The key functional roles of a healthy GM [18]

2.4.1 Colonization resistance

One of the key protective functions of a diverse and balanced GM is colonization resistance. This process helps prevent the establishment and overgrowth of potentially harmful bacteria. Colonization resistance involves several strategies that maintain stable populations of resident microbes.

For example, gut bacteria compete for nutrients and ecological niches, limiting the growth of invading or opportunistic organisms. Additionally, many beneficial microbes produce antimicrobial compounds that help control the surrounding bacterial populations. Bacteriocins, which are diffusible proteins secreted by certain species, can have strong toxic effects on other bacteria, including closely related strains. They achieve this by disrupting cell membranes, degrading nucleic acids, or interfering with cell wall synthesis.

Members of the Bacteroidetes and Proteobacteria phyla also have specialized antibacterial secretion systems that can target genetically different neighboring cells. Although the specific mechanisms can vary among different bacterial groups, the overall aim of colonization resistance is to maintain a stable and healthy gut microbial ecosystem [18].

2.4.2 Immune modulation

The GM contributes to both innate and adaptive immune regulation through multiple complex and only partially elucidated mechanisms. Broadly, the interaction between commensal microorganisms and the host immune system modulates the balance between pro- and anti-inflammatory responses within the intestinal environment. In a state of equilibrium, resident microbes assist the immune system in distinguishing between harmless commensals and potentially harmful pathogens, ensuring that inflammation is appropriately activated or suppressed. Pattern recognition receptors, such as TLRs on intestinal epithelial cells, play a pivotal role in this process by recognizing MAMPs and helping the host discriminate between familiar and foreign microbes. Commensal bacteria also promote the production of IgA, which serves as a key line of defense at mucosal surfaces, and influence the differentiation of T lymphocytes, maintaining a balance between effector T helper cells and immunoregulatory T cells (Tregs). Certain bacterial taxa, including *Clostridia* (Firmicutes), *Bacteroides fragilis* (Bacteroidetes), and *Bifidobacterium infantis*, have been shown to stimulate Treg recruitment and expansion, supporting immune tolerance. Additionally, species such as *Faecalibacterium prausnitzii* are associated with a reduction in pro-inflammatory cytokines, including interleukin-12 (IL-12), alongside an increase in anti-inflammatory cytokines like IL-10. Collectively, these interactions highlight how the GM not only facilitates effective immune activation during infection but also suppresses excessive or inappropriate inflammation, thereby preserving intestinal epithelial integrity and overall immune homeostasis [18].

2.4.3 Bile acid homeostasis

Bile acids, synthesized in the liver and secreted into the small intestine, play a crucial role in lipid digestion and the absorption of fat-soluble vitamins. Primary bile acids are initially released in conjugated form; however, several commensal bacteria—including members of the Firmicutes (e.g., *Clostridium*, *Lactobacillus*, *Enterococcus*), Bacteroidetes (e.g., *Bacteroides*), and Actinobacteria (e.g., *Bifidobacterium*) phyla—express bile salt hydrolase (BSH) enzymes capable of deconjugating these molecules back into their unconjugated state. This enzymatic process facilitates the enterohepatic recirculation of the majority of the bile acid pool. A small fraction of primary bile acids that reach the colon are subsequently converted into secondary bile acids through microbial 7α-dehydroxylation, a transformation carried out by a limited subset of gut microbes (<0.025% of the total community), among which *Clostridium scindens* is the best characterized. Both primary and

secondary bile acids contribute to glucose metabolism and possess antimicrobial detergent properties that reinforce colonization resistance. Notably, secondary bile acids are particularly effective in suppressing *Clostridioides difficile* growth. Because of the microbiota's central role in bile acid transformation, the ratio of secondary to primary bile acids in the colon is increasingly recognized as a potential biomarker for distinguishing a balanced, healthy microbiome from a dysbiotic one [18].

2.4.4 Short-chain fatty acid production

GM ferment unabsorbed starches and soluble dietary fiber from the host's diet, which results in generation of SCFA. Diets that are rich in fiber (e.g., plant-based) and fermented foods are therefore considered "gut healthy" because they are sources of SCFA. The most predominant SCFA are butyrate, propionate, and acetate. SCFA production is widespread among microbiota, with generation of specific SCFA varying among specific bacterial groups. Butyrate generation is particularly important as it is the preferred energy source of colonic epithelial cells. Several members of the Firmicutes phylum (e.g., *Roseburia* and *Ruminococcus* strains) are key butyrate producers. Butyrate produced by GM is estimated to provide 5–10% of caloric requirements for the host by supplying energy to colon cells [67,68].

SCFA are also believed to be involved in other functions, including appetite and glucose tolerance [67]. Furthermore, SCFA have been shown to have various immune-modulating properties, including antagonizing the proinflammatory effects of certain cytokines on the gut epithelium and the ability to interact with neutrophils to either stimulate or suppress local activity. SCFA are therefore important contributors to gut barrier health by both providing energy to epithelial cells and tempering local inflammation. These functions support the purported health benefits of a high-fiber diet and, conversely, the potential loss of benefits associated with a traditional Western diet [18].

2.4.5 Vitamin synthesis

The intestinal microbiota plays a crucial role in the endogenous synthesis of several vitamins essential for host metabolism. Certain B vitamins, including cyanocobalamin (vitamin B12), are produced exclusively by specific gut bacterial taxa such as members of the Firmicutes,

Actinobacteria, and Proteobacteria phyla. These vitamins are vital for numerous cellular functions, including DNA synthesis, repair, and overall metabolic regulation. In addition, several commensal species—such as *Bacteroides fragilis*, *Eubacterium lentum*, *Enterobacter agglomerans*, *Serratia marcescens*, and *Enterococcus faecium*—are known to synthesize vitamin K, which is required for normal coagulation processes and bone health. Consequently, alterations in the abundance or activity of these bacterial populations may influence the bioavailability of endogenously produced vitamins, thereby impacting host metabolic homeostasis [18].

2.5 Dysbiosis

The human gastrointestinal tract harbors a highly complex microbial ecosystem that plays a pivotal role in host physiology (**Figure 8**). In addition to supporting digestion and energy extraction, the GM is fundamental for maintaining immune balance. Commensal microbes contribute to the differentiation of Tregs and Th17 cells, modulate the production of both pro- and anti-inflammatory cytokines, and support intestinal barrier integrity by enhancing tight junctions, stimulating mucus and antimicrobial peptide secretion, and regulating epithelial signaling. These functions create a tightly controlled network that protects the host against pathogens while preventing excessive inflammation [69,70].

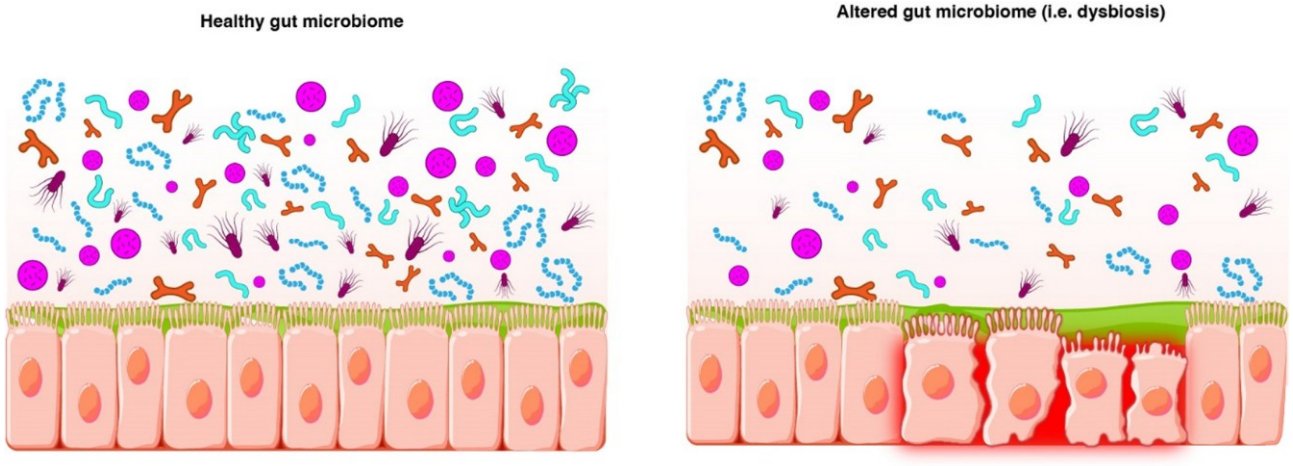

Figure 8. Schematic representation of a healthy and an altered gut microbiome [18]

Dysbiosis refers to the disruption of this balance and is typically characterized by a loss of beneficial microbes, an overgrowth of potentially pathogenic taxa such as Proteobacteria (including *Escherichia coli* and *Klebsiella* spp.), and a reduction in overall microbial diversity. In healthy individuals, *Proteobacteria* usually account for less than 10% of the GM, whereas in dysbiotic states their relative abundance may rise to 20–30%[18] .

Such alterations compromise the production of key microbial metabolites, including SCFAs, secondary bile acids, and tryptophan-derived compounds, all of which exert immunomodulatory functions. SCFAs, for instance, promote Treg differentiation, suppress pro-inflammatory cytokine secretion, and strengthen epithelial barrier function. Conversely, reduced SCFA levels, along with disturbances in bile acid metabolism and tryptophan catabolites, negatively affect both innate and adaptive immune responses. Dysbiosis also increases host exposure to LPS and other PAMPs, which activate TLRs and NOD-like receptors (NLRs), initiating inflammatory cascades that can propagate locally and systemically [71,72].

The onset of dysbiosis is driven by a multifactorial interplay of host genetics, diet, antibiotic exposure, infections, stress, and chemical insults. Although the GM shows remarkable resilience and adaptability, the cumulative impact of several perturbations can push the system beyond a tipping point, resulting in persistent microbial imbalance. Additional factors, including bacteriophages, bacteriocins, and oxidative stress, may exacerbate these changes and further disrupt host immune regulation [71].

Clinically, dysbiosis has been implicated in a broad spectrum of conditions, including inflammatory bowel diseases, metabolic syndrome, obesity, type 2 diabetes, and systemic immune dysfunction. These associations likely reflect the downstream effects of altered microbial metabolites on cytokine signaling, intestinal barrier integrity, and systemic inflammation, highlighting the functional relevance of the microbiota–immune axis for human health [69–72].

Despite its clinical importance, no universally accepted gold standard exists for the identification of dysbiosis. Several indices have been proposed, most of which were first developed in the context of recurrent *Clostridioides difficile* infection (CDI), a condition strongly linked to microbiome disruption. One such index is based on the ratio of Proteobacteria strains to the overall bacterial strains, with higher values suggesting a dysbiotic state. Another, the Microbiome Health Index (MHI), evaluates microbial balance across the Firmicutes, Bacteroidetes, and Proteobacteria phyla. Specifically, the MHI considers the relative abundance of taxa associated with intestinal homeostasis, such as *Clostridia* (phylum Firmicutes) and *Bacteroidia* (phylum Bacteroidetes), and compares them with

taxa more commonly linked to pathogenesis, including *Bacilli* (phylum Firmicutes) and *Gammaproteobacteria* (phylum Proteobacteria) [18].

In addition to its well-established role in CDI, dysbiosis has been implicated in a wide range of pathological conditions across different organ systems. Importantly, whether dysbiosis acts as a cause or a consequence of these conditions is often difficult to discern, highlighting the bidirectional nature of the relationship between microbiome alterations and disease. This dynamic has been particularly discussed in the context of inflammatory bowel diseases, but also in neurological and psychiatric disorders, where dysbiosis may contribute to disruptions of the gut–brain axis. In several cases, proposed mechanisms involve compromised intestinal barrier integrity, or so-called “leaky gut”, whereby localized inflammation increases host exposure to luminal antigens, triggering both local and systemic immune responses.

The mechanistic links between dysbiosis and disease are likely multifactorial and remain incompletely understood. Beyond its role in disease susceptibility, dysbiosis also appears to influence the pharmacokinetics and pharmacodynamics of several drugs. For instance, the immunosuppressant tacrolimus can be metabolized by gut microbes into less active derivatives, potentially explaining the variability in patient responses to oral therapy. Similarly, the antihyperglycemic efficacy of metformin is significantly reduced when commensal bacteria are depleted by concomitant oral vancomycin administration. Other well-documented examples include the inactivation of digoxin by *Eggerthella lenta* and the altered activity of vitamin K antagonists such as warfarin in patients receiving antibiotics that suppress vitamin K–producing bacteria. More recently, evidence has also suggested that the clinical response to immune checkpoint inhibitors in oncology may be enhanced by supplementation with selected commensal species.

Altogether, these findings underscore the emerging role of the GM in drug metabolism and therapeutic efficacy. Although the precise mechanisms remain to be clarified, it has been postulated that bacterial classes such as *Clostridia* and *Bacilli* may influence the expression of host transporters, including P-glycoprotein, as well as receptors regulating key metabolic systems such as cytochrome P450. This raises the possibility that dysbiosis not only predisposes to disease but also modifies host–drug interactions, with significant implications for personalized medicine [18].

2.6 Gut microbiota and disease

Alterations in the composition and function of the GM can disrupt its ability to support host health, potentially favoring the expansion of opportunistic microbes and leading to an imbalance in microbial metabolites. Such perturbations have been implicated in a wide spectrum of local, systemic, and distant-organ pathologies (**Table 2**), some of which may be amenable to microbiome-targeted therapeutic interventions [73].

Disease categories	**Specific diseases**	**Associated dysbiotic features**
Immune-mediated/autoimmune diseases	Inflammatory Bowel Disease (IBD)	- Increase in virulent gut microbes (*Enterobacteriaceae* species, *Bacteroides fragilis*) and mucolytic *Ruminococcus* sp. - Decrease in butyrate-producing *Firmicutes* (*Faecalibacterium prausnitzii*, *Roseburia hominis*)
	Irritable Bowel Syndrome (IBS)	- Increase in *Escherichia coli* - Decrease in *Clostridium leptum* group of bacteria and *Bifidobacterium* - Decrease in bile acid biotransformation
	Celiac Disease	- Increase in *Bacteroides-Prevotella* group - Decrease in *Bifidobacterium* - Varying observation (decrease or no change) in *Clostridium Histolyticum*, *C. lituseburense*, and *Faecalibacterium prausnitzii* - Alteration in SCFAs composition, but overall increase in total SCFA
	Systemic Lupus Erythematosus (SLE)	- Increase in *Blautia* sp. And Gram-negative bacteria such as *Proteobacteria* - Decrease in GM diversity, *Odoribacter* sp., *Alistipes* sp.

		- Increase in serum endotoxin
	Type-1 Diabetes	- Increase in *Bacteroidetes* - Decrease in *Actinobacteria*, *Firmicutes*, and *Firmicutes*/*Bacteroidetes* ratio
	Reumatoid Arthritis (RA)	- Increase in *Prevotella copri* and decrease in *Bacteroides* sp. In new-onset RA - Increase in microbiota diversity of *Lactobacillus* genus in early RA
	Atopic Disease (E.g., childhood allergic asthma)	- Increase in fecal burden of *Clostridium difficile*, and *C. difficile*/*Bifidobacteria* ratio
Metabolic disorders/cardiovascular disorders	Obesity	- Increase in Firmicutes, Actinobacter - Fluctuating trends observed in Bacteroidetes (decrease, stable, or increase) - Increase in glycoside hydrolase and SCFAs (butyrate and acetate)
	Type-2 Diabetes	- Increase in *Lactobacillus* - Decrease in *Clostridium coccoides*, *Atopobium cluster*, and *Prevotella* - Decrease in butyrate biosynthesis
	Hypertension	- Increase in the Firmicutes/Bacteroidetes ratio, lactate-producer - Decrease in microbiota diversity, acetate- and butyrate-producers
	Atherosclerosis	- Increase in metabolites TMAO, endotoxin level (risk factor for early atherosclerosis)
Cancer	Colorectal Cancer (CRC)	- Increase in enterotoxigenic *Bacteroides fragilis*, and pathobionts *Fusobacterium* and *Campylobacter* sp. - Decrease in butyrate-producer (*Faecalibacterium* and *Roseburia*)

Neuropsychiatric	Autism Spectrum Disorder (ASD)	- Increase in *Clostridium* sp., *Bacteroidetes*, *Lactobacillus*, *Desulfovibrio* - Decrease in *Bifidobacteria*
	Alzheimer's Disease	- Link between microbial amyloids, LPS, GABA, and age-related gut/brain barrier permeability
	Depression	- Increase in genus *Eggerthella*, *Holdemania*, *Gelria*, *Turicibacter*, *Paraprevotella*, *Anaerofilum* - Decrease in GM diversity, *Prevotella* and *Dialister*
	Parkinson's Disease (PD)	- Decrease in butyrate-producing genera (*Blautia, Coprococcus, Roseburia*) with rise in mucosal Proteobacteria - Increased gene expression in LPS biosynthesis and microbial type III secretion system
Infectious disease	*Clostridium difficile* infection (CDI)	- Increase in *Clostridium difficile* - Decrease in GM diversity and secondary bile acids-producing *Clostridium scindens*

Table 2. Overview of representative diseases linked to gut microbiota dysbiosis, indicating typical increases or decreases in specific microbial taxa and associated alterations in metabolic or inflammatory functions.

2.7 Gut microbiota and nutrition

Diet is a key determinant of microbial diversity, as dietary patterns exert a profound influence on the composition and functionality of the GM. Diets rich in fiber, polyphenols, and fermented foods promote microbial diversity and the proliferation of beneficial taxa such as *Bifidobacterium* and *Lactobacillus*. Conversely, Western-style diets high in saturated fats, refined sugars, and low in fiber are associated with dysbiosis—an imbalance in microbial communities linked to

inflammation, metabolic disorders and disruptions in estrogen metabolism—key factors implicated in the pathogenesis of breast cancer (BC). Gut microbes ferment dietary fibers into SCFAs like acetate, propionate, and butyrate. These metabolites regulate intestinal barrier integrity, modulate immune responses, and influence energy homeostasis. Butyrate, in particular, serves as a primary energy source for colonocytes and exhibits anti-inflammatory properties [74]. Emerging evidence supports the concept of personalized nutrition based on individual microbiome profiles. Interindividual variability in microbial composition can influence glycemic responses, lipid metabolism, and nutrient absorption, suggesting that tailored dietary interventions may optimize health outcomes and exert protective effects against carcinogenesis. In a study of Wastyk et al., a randomized longitudinal intervention in healthy adults showed that dietary modulation of the gut microbiome elicits distinct effects on host immunity, with different diet patterns differentially shaping microbial composition and diversity and influencing inflammatory and immune-related outcomes[75].

Advancements in metagenomics and metabolomics are enhancing our understanding of diet–microbiota–host interactions. These tools enable high-resolution profiling of microbial communities and their functional capacities, paving the way for microbiome-informed dietary guidelines and therapeutic strategies [76].

2.8 Gut microbiota and physical activity

Physical activity plays a significant role in shaping the composition and functionality of the GM, contributing to overall health and well-being. Regular exercise has been shown to increase microbial diversity and promote the growth of beneficial bacteria, such as *Akkermansia muciniphila* and *Faecalibacterium prausnitzii*, which are associated with anti-inflammatory effects and improved intestinal barrier integrity. These changes in microbial populations are not merely local; they influence systemic processes, including immune modulation and metabolic regulation.

One of the key mechanisms through which physical activity exerts its effects is the enhancement of SCFA production. SCFAs, such as butyrate, propionate, and acetate, are microbial metabolites that support colonocyte health, regulate immune responses, and contribute to energy homeostasis. The increased availability of these compounds following regular exercise may help reduce systemic inflammation and improve insulin sensitivity, offering protection against metabolic disorders.

Importantly, the impact of physical activity on the GM appears to be dose-dependent. The effects of physical activity on the gut microbiome are influenced by both the type and intensity of exercise performed. Moderate, consistent exercise tends to foster a favorable microbial environment and support immune function whereas excessive or high-intensity training—especially without adequate recovery—may lead to gastrointestinal disturbances and systemic inflammation, potentially leading to microbial imbalance [77]. This underscores the importance of tailoring physical activity to individual needs and capacities.

Notably, combining aerobic and resistance training has been shown to significantly enhance microbial diversity, a factor associated with reduced incidence of chronic metabolic diseases. Exercise also contributes to increased production of SCFAs, improved nutrient absorption, and modulation of neuroendocrine pathways, all of which help strengthen gut barrier function.

Emerging research also suggests that the GM may influence athletic performance and recovery. Certain microbial profiles have been linked to enhanced endurance, reduced oxidative stress, and improved nutrient absorption, indicating a bidirectional relationship in which not only does exercise shape the microbiota, but the microbiota may also modulate physical performance [78]. Recent studies also suggest that physical activity can beneficially modulate the GM , which in turn may influence cancer risk and outcomes. This relationship is particularly relevant in the context of systemic inflammation, immune regulation, and treatment tolerance [79].

3. Decoding the microbial signatures of Parkinson's Disease: from motor symptoms to gut microorganisms

3.1 Background

The GM plays a fundamental role in maintaining host homeostasis, and its imbalance, or dysbiosis, has been associated with a wide spectrum of chronic diseases. Growing evidence indicates that the GM communicates with the central nervous system through the gut–brain axis, influencing neural, endocrine, and immune pathways. This bidirectional interaction has become a focus of interest in the context of neurodegenerative disorders, particularly PD and Alzheimer's disease (AD), where alterations of the GM are increasingly recognized as potential contributors to disease onset and progression [80].

Although the notion of gut–brain communication has historical roots dating back to the eighteenth century, only in recent decades has the microbiome been identified as a central component of this axis. Advances in neurogastroenterology and microbiome research have since revealed that disruptions in this system are implicated in a range of neurodevelopmental, psychiatric, and neurological conditions. Among these, PD has emerged as the most extensively studied, positioning it as a paradigmatic example of how microbiota–brain interactions may shape neurodegenerative disease processes [81].

3.1.1 Parkinson's disease

PD is a progressive neurodegenerative disorder and one of the most prevalent neurological conditions worldwide [82].

The pathophysiology of PD is primarily characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta, a region in the midbrain. This neuronal loss is often accompanied by the abnormal accumulation of misfolded α-synuclein proteins, which form inclusions known as Lewy bodies (LBs) [83].

Although the exact cause of idiopathic PD remains unclear, several risk factors have been consistently identified. These include aging, genetic predisposition, exposure to pesticides, and environmental toxins [84].

Clinically, PD manifests through a wide range of motor and non-motor symptoms. The key motor features are resting tremor, muscular rigidity, bradykinesia (slowness of movement), and postural instability. Together, these symptoms lead to progressive difficulties in motor control and mobility. Additionally, patients with PD often experience various non-motor complications, such as neuropsychiatric disturbances (including depression and anxiety), cognitive decline that can lead to dementia, and autonomic dysfunctions, such as orthostatic hypotension and abnormal sweating.
These combined motor and non-motor symptoms significantly impact patients' quality of life, emphasizing the complex and multifaceted nature of the disease [83].

3.1.2 The gut-brain axis theory in Parkinson's disease

The concept of communication between the gastrointestinal tract and the central nervous system (CNS) has a long intellectual history. As early as the eighteenth century, scholars began to propose a close relationship between digestion, emotions, and identity [85] . In 1765, the Scottish physician Robert Whytt introduced the term *"nervous sympathy"*, describing a functional network by which the brain and internal organs, including the gut, were interconnected. This idea anticipated the recognition that disturbances in visceral function could influence mood and behavior [86].
During the nineteenth century, the anatomical underpinnings of this bidirectional system were progressively delineated [85]. Research on the vagus nerve and the enteric nervous system demonstrated the existence of direct neural pathways linking the gut and the brain. These discoveries laid the foundation for the modern concept of the gut–brain axis.
In the late twentieth century, interest in the gut–brain connection was revitalized by advances in neurogastroenterology, particularly with the characterization of the enteric nervous system as the "second brain". At the same time, experimental studies began to highlight the role of gut-derived signaling molecules, including hormones and neurotransmitters, in influencing CNS function.
The early twenty-first century marked a turning point with the recognition of the gut microbiome as a fundamental third component of this axis. The advent of high-throughput sequencing technologies enabled detailed characterization of the human microbiota, revealing its ability to modulate neural, immune, and endocrine pathways. From this point onwards, the microbiome–gut–brain axis became a major focus of biomedical research, with accumulating evidence linking its alterations to neurodevelopmental, psychiatric, and neurodegenerative disorders. Among these, PD has emerged as one of the most extensively investigated models [87–91].

Gastrointestinal dysfunctions, including constipation, dysphagia, and nausea, are common in PD and may precede motor symptoms by more than 20 years. Since the early 20th century, Lewy pathology—characterized by α-synuclein aggregation—has been observed not only in the CNS but also in the peripheral and enteric nervous systems (ENS). Building on these findings, Braak's hypothesis (2003) proposed that PD may originate in the gut, with α-synuclein pathology spreading to the brain via enteric neurons (**Figure 9**) [81].

Subsequent postmortem and biopsy studies confirmed widespread α-synuclein deposits in the myenteric and submucosal plexus, detectable decades before diagnosis, and particularly enriched along vagal innervation. Evidence from prodromal cohorts, such as patients with isolated REM sleep behavior disorder (iRBD), further supports the presence of early gastrointestinal α-synuclein pathology and dysfunction preceding CNS involvement. Although some studies reported inconsistent results, methodological variability likely explains these discrepancies.

Experimental models have provided strong mechanistic support, showing that α-synuclein aggregates can propagate from the gut to the CNS along the vagus nerve in a time-dependent manner, recapitulating both non-motor and motor features of PD. Vagotomy appears protective, while hemivagotomy or sympathectomy block propagation in animal models. Importantly, human postmortem studies demonstrate Lewy pathology in sympathetic ganglia, the heart, and spinal cord, sometimes even in the absence of CNS pathology. These findings collectively suggest that PD pathology may, in some cases, originate in the gut and spread retrogradely to the brain through both vagal and sympathetic routes [92].

While different origins and spreading routes of pathological α-synuclein explain part of PD heterogeneity, they do not clarify the initial trigger of gut pathology. In the past decade, alterations in the gut microbiome have gained attention as potential contributors to PD pathogenesis. Preclinical data strongly suggest a causal role, though in humans it remains unclear whether dysbiosis represents a primary driver or a secondary consequence of disease and treatment.

In animal models, germ-free conditions or antibiotic depletion reduced α-synuclein inclusions, neuroinflammation, and motor deficits, whereas fecal microbiota transplantation (FMT) from PD patients exacerbated motor impairment compared to FMT from healthy donors. Conversely, FMT from healthy animals ameliorated both motor and gastrointestinal dysfunction, supporting a direct pathogenic role of gut dysbiosis.

Human case–control studies since 2015, including multiple meta-analyses, consistently report depletion of SCFA-producing bacteria (e.g., *Roseburia*, *Faecalibacterium*, *Prevotella*) and reduced

fecal SCFA levels in PD patients. Given their role in intestinal barrier integrity and anti-inflammatory signaling, SCFA depletion may promote gut permeability and inflammation, features repeatedly observed in PD. However, findings in prodromal cohorts remain inconsistent. Other recurrent observations include enrichment of *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*. Their interpretation remains debated: Lactobacillus and Bifidobacterium are generally considered beneficial, but may metabolize levodopa, suggesting treatment-driven selection. *Akkermansia muciniphila*, typically linked to metabolic health, is paradoxically increased in PD and iRBD, possibly as a compensatory response to microbiome shifts, although mechanistic studies indicate it may enhance α-synuclein aggregation.

Environmental exposures, such as pesticides or trichloroethylene, and gastrointestinal comorbidities like IBD, induce microbiome changes resembling those observed in PD, lending plausibility to a primary role of dysbiosis. Still, aging, genetics, and host factors must be considered. Emerging longitudinal studies suggest microbiome composition may also influence clinical heterogeneity and progression. For example, reduced *Prevotella* abundance correlates with faster progression, while lower baseline Bifidobacterium has been linked to worsening neuropsychiatric symptoms. These findings highlight the potential of microbiome-targeted interventions as disease-modifying strategies, although larger and longer follow-up studies are required to establish causality.

Accumulating evidence implicates gut dysbiosis in PD pathogenesis, with alterations in microbial composition—particularly depletion of SCFA-producing taxa and enrichment of *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*—suggested to influence barrier integrity, inflammation, and α-synuclein aggregation. Yet, microbiome-related mechanisms are unlikely to act in isolation. Increasing attention has turned to another gut–brain communication route, involving EECs, vagal afferents, and the brainstem.

EECs are specialized chemosensory cells capable of transducing microbial and nutritional signals into neuronal and hormonal outputs. They display neuronal-like properties, express α-synuclein, and respond to bacterial components with increased intracellular α-synuclein, suggesting a potential initiating site for pathology in the presence of gut dysbiosis. Functional alterations of EECs have been documented in PD, most notably reduced postprandial secretion of glucagon-like peptide-1 (GLP-1), a key L-cell hormone. Because SCFAs stimulate GLP-1 release via Free Fatty Acid Receptor 2 (FFAR2) signaling, depletion of SCFA-producing bacteria in PD may directly impair GLP-1 responses.

Beyond its metabolic role, GLP-1 exerts broad neuroprotective and anti-inflammatory effects, with preclinical studies showing that GLP-1 receptor agonists (GLP-1RAs) prevent dopaminergic neurodegeneration, preserve striatal dopamine, and reduce microglial activation in PD models. These findings have spurred clinical trials testing GLP-1RAs in PD patients.

Taken together, alterations of both the gut microbiome and the enteroendocrine system highlight complementary pathways through which gut-derived signals may influence PD pathogenesis and progression, opening the way to microbiome- and GLP-1–based therapeutic strategies [93].

3.1.3 Gut microbiota state in Parkinson's disease

Aging is the most significant risk factor for PD, and the biochemical changes that accompany aging worsen the pathological abnormalities observed in the brains of individuals with PD [94]. The primary event in the disease is the progressive dysfunction and loss of DA neurons in the substantia nigra. This loss ultimately leads to impaired activation and function of motor cortex neurons, resulting in motor symptoms [95,96]. However, the neuropathological changes in PD are not confined to the nigrostriatal system; abnormalities have also been found in the autonomic nervous system, olfactory bulb, lower brainstem, and cerebral cortex [97]. These widespread alterations contribute to a variety of non-motor symptoms that occur alongside PD, including sleep disturbances, cognitive impairment, and psychiatric symptoms, in addition to the motor features associated with the disease [98].

Among the non-motor symptoms, gastrointestinal dysfunction—particularly constipation—affects nearly 80% of patients and often appears years before the onset of motor symptoms. Indeed, idiopathic constipation has been identified as a significant risk factor for PD, indicating that peripheral pathophysiological processes may play an early role in the onset of the disease.

Neuropathological studies have shown that LBs, which are primarily composed of misfolded α-synuclein fibrils, and Lewy neurites, which are elongated inclusions in axons and dendrites, are characteristic lesions of PD. Under normal conditions, α-synuclein is a soluble monomer predominantly expressed in presynaptic terminals, where it moderates vesicle trafficking and regulates DA release. However, oligomeric and protofibrillar forms of α-synuclein are highly neurotoxic as they disrupt synaptic vesicle dynamics, interfere with the Soluble N-ethylmaleimide–Sensitive Factor Attachment Protein Receptor (SNARE) complex, decrease dopamine transporter (DAT) expression, and impair DA release. These early changes in presynaptic function lead to axonal

dysfunction and synaptic disconnection, which occur before visible neuronal loss. As the disease progresses, widespread degeneration of axons, synaptic terminals, and neuronal cell bodies becomes apparent [99].

Mechanistically, the death of DA neurons may occur through apoptosis, with factors such as mitochondrial dysfunction, oxidative stress, altered protein degradation, and inflammation all playing roles in the degenerative process. Dysregulation of mitochondrial dynamics, particularly through the proteins Dynamin-Related Protein 1 (DRP1) and Optic Atrophy 1 (OPA1), promotes the release of cytochrome C and the activation of caspase-9, leading to apoptotic cell death. These molecular events position mitochondria at the center of PD pathogenesis [100].

The Braak hypothesis suggests that PD may begin in the periphery, particularly in the GIT, with pathological α-synuclein ascending to the brain via vagal pathways [101–103]. Research indicates that the ENS and the dorsal motor nucleus of the vagus nerve are affected early in the disease, sometimes even before the substantia nigra. Experimental studies have shown that when α-synuclein is injected into the intestinal wall of rats, it can be retrogradely transported to the brain, providing strong support for the gut-origin hypothesis. Furthermore, α-synuclein has been demonstrated to propagate between neurons through endocytosis, mimicking prion-like mechanisms of transmission [104].

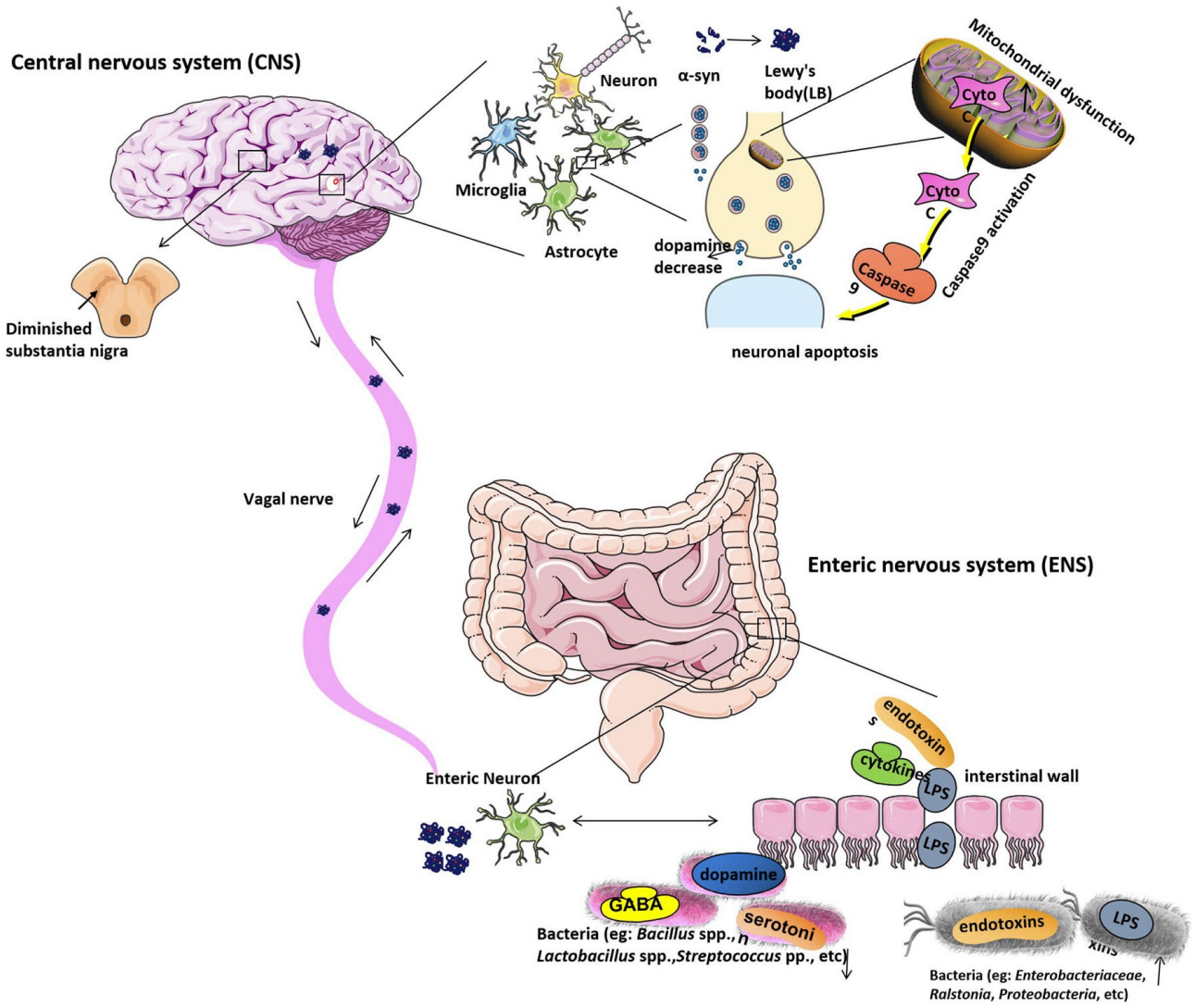

Figure 9. Bidirectional communication along the gut–brain axis, showing interactions between the enteric nervous system, gut microbiota, and central nervous system via microbial metabolites, cytokines, endotoxins, and vagal signaling [104]

In recent years, there has been increasing attention on the role of GM in this process. Dysbiosis has been consistently observed in PD patients compared to healthy controls and is believed to contribute to the abnormal aggregation of α-synuclein within the ENS. Neuropathological evidence indicates that colonic biopsies from PD patients almost invariably contain α-synuclein deposits in both the Meissner and Auerbach plexuses. Supporting this gut-brain axis model, epidemiological studies have shown that complete vagotomy reduces the risk of developing PD by about 50% in large Danish and Swedish cohorts. In experimental models, the administration of misfolded α-synuclein—whether intraperitoneally in PD mice or directly into the gastric wall of wild-type mice—

results in pathological spreading to the substantia nigra, replicating the key features of the human disease [105].

Mechanistic studies reveal how alterations in GM may exacerbate PD pathology. A deficiency in dietary fibers promotes the expansion of *Akkermansia muciniphila*, a mucin-degrading bacterium that thins the intestinal mucus layer and increases gut permeability. At the same time, butyrate-producing bacteria such as *Faecalibacterium* and *Roseburia* are reduced in PD, leading to diminished production of SCFAs. This depletion not only compromises the intestinal barrier but also enhances the translocation of luminal toxins, such as LPS and pesticides, into the ENS, where they may trigger α-synuclein fibrillization. Under normal conditions, SCFAs exert powerful anti-inflammatory effects by inhibiting histone deacetylases, promoting Treg differentiation, and stimulating the release of anti-inflammatory cytokines. Their reduction amplifies neuroinflammation both peripherally and centrally. Additionally, SCFAs like acetate and propionate activate G-protein–coupled receptors (GPR41 and GPR43), which regulate satiety and gastrointestinal motility through the secretion of peptide YY (PYY) and GLP-1. Disruption of these signaling pathways contributes to the significant gastrointestinal dysfunction observed in PD [106].

Overall, alterations in the composition or activity of gut microbes may weaken the intestinal barrier and facilitate the transmission of pathogenic proteins from the gut to the brain, resembling prion-like propagation. The gut-brain axis is closely linked to neuroinflammation, as microbial metabolites influence α-synuclein aggregation, regulate immune responses, and modulate protein toxicity, even when their primary molecular targets differ. This perspective highlights the complex and reciprocal interactions between GM and neurodegenerative processes, particularly in relation to PD and prion disorders. Ultimately, dysbiosis, mitochondrial dysfunction, and α-synuclein pathology converge to drive dopaminergic neuronal loss, striatal dopamine depletion, and the emergence of the characteristic motor and non-motor symptoms of PD (**Figure 10**) [107].

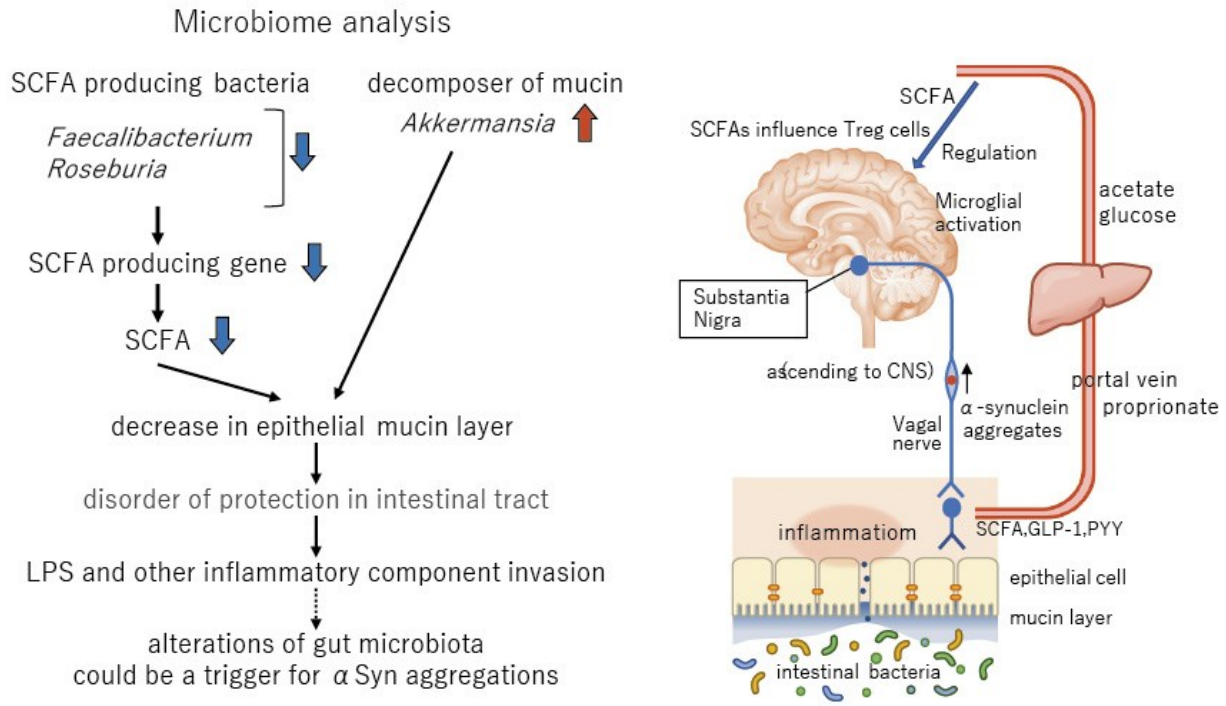

Figure 10. Putative mechanisms linking gut dysbiosis to the development and progression of Parkinson's disease (PD) [108]

3.1.4 Microbiota-drug interactions in Levodopa treatment of Parkinson's Disease

Levodopa (L-dopa) remains the cornerstone of pharmacological therapy for PD, as it serves as a metabolic precursor of dopamine capable of crossing the blood–brain barrier (BBB). Once in the central nervous system, it is converted into dopamine by the enzyme aromatic amino acid decarboxylase (AADC), thereby alleviating the motor symptoms associated with dopaminergic neurodegeneration in the substantia nigra. However, a substantial portion of levodopa undergoes premature decarboxylation in peripheral tissues, particularly within the gastrointestinal tract, where the dopamine formed cannot cross the BBB and contributes to adverse systemic effects. Although peripheral AADC inhibitors such as carbidopa and benserazide are routinely co-administered to enhance levodopa bioavailability, intestinal metabolism remains a major limiting factor in therapeutic efficacy.

Recent studies have identified a pivotal role of the GM in this process. Certain intestinal bacteria—most notably *Enterococcus faecalis* and *Lactobacillus* species—express tyrosine decarboxylase (TyrDC), an enzyme capable of converting L-dopa into dopamine within the gut lumen. This

microbial decarboxylation further reduces the amount of L-dopa available for absorption and transport to the brain. Moreover, the abundance of the TyrDC gene in the fecal microbiota of PD patients has been correlated with increased levodopa dosage requirements and reduced treatment responsiveness. These findings suggest that microbial metabolism represents a critical yet underappreciated determinant of L-dopa pharmacokinetics, highlighting the GM as a potential therapeutic target to improve drug efficacy and reduce systemic side effects in PD (**Figure 11**) [109].

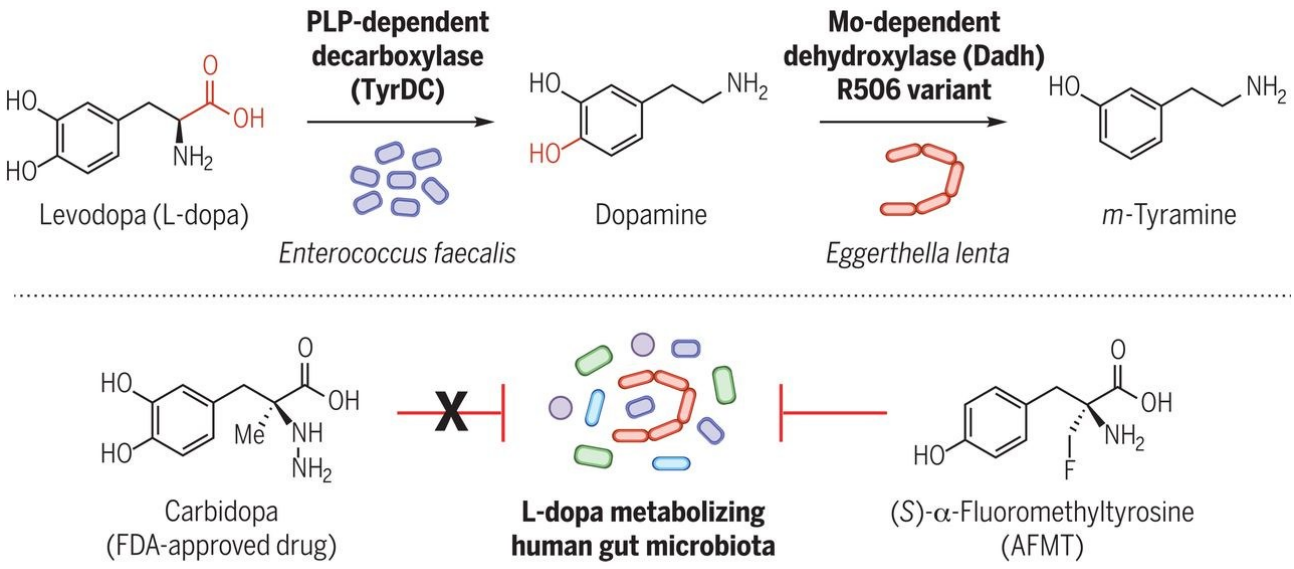

Figure 11. L-dopa metabolism by bacterial and human enzymes [110]

3.2 Methods

3.2.1 Study design and subjects

This research was conducted as part of the *MARKERS-NDD* study, a prospective, observational, longitudinal investigation designed to collect clinical, biological, and instrumental data from patients with neurodegenerative diseases undergoing routine follow-up assessments within standard clinical practice. The study was approved by the local Ethics Committee, registered on clinicaltrial.gov (NCT06596746) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study.

Patients were diagnosed with PD according to the United Kingdom (UK) Parkinson's Disease Society Brain Bank [111].

Total 109 PD patients were recruited from the Clinical Trial Centers (CTCs) for Parkinson's Disease of IRCCS San Raffaele Roma.

The following data were collected for all patients (**Table 3**): gender, year of birth, disease duration, antiparkinsonian drugs and concomitant medications, Levodopa Equivalent Daily Dose (LEDD), presence or absence of motor fluctuations, disease severity based on Hoehn and Yahr (H&Y) scale, and Movement Disorder Society–Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS III) score.

Variables	**Non-fluctuating PD (n=74)**		**Fluctuating (n=35)**	
	% (n) / median [IQR]	**p-value**	**% (n) / median [IQR]**	**p-value**
Sex (% males)	50 (37)		60 (21)	
Age	63 [57.25-69]	0.388	70 [61-74]	0.548
Disease duration	2 [1-4.75]	<0.001	13 [8-17]	0.199
LEDD	300 [150-400]	0.011	940 [802.5-1100]	0.439
H&Y	2 [1.625-2]	<0.001	2.5 [2-3]	<0.001
MDS-UPDRSIII	19 [13.25-26]	0.009	32 [22-38]	0.223

Table 3. Clinical variables in non-fluctuating and fluctuating patients

3.2.2. Clinical assessment

PD-related clinical scales were collected from each participant during their outpatient visit. These measures included: disease duration, defined as the time interval between onset of typical PD motor symptoms and the date of the biospecimen collection; the LEDD, calculated as a sum of all antiparkinsonian medications multiplied by their respective conversion factors, as previously described [112]; presence or absence of motor fluctuations; H&Y stage, assessed in the ON phase for fluctuating patients, which ranges from 1 to 4 and reflects disease severity (**Table 4**) [113]; and the MDS-UPDRS III, corresponding to the motor examination (**Table 5**) [114].

Stage	Symptoms
1	Unilateral involvement only
1.5	Unilateral and axial involvment
2	Bilateral involvement without impairment of balance
2.5	Mild bilateral involvement without recovery on retropulsion (pull) test
3	Mild to moderate bilateral involvement, some postural instability but physically independent
4	Severe disability, still able to walk and to stan unassisted

Table 4. Hoehn and Yahr (H&Y) scale

MDS-UPDRS part III score	Motor Symptoms
0-15	Mild motor symptoms
16-30	Moderate motor symptoms
31-45	Severe motor symptoms
46-52	Extremely severe motor symptoms

Table 5. Movement Disorder Society–Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS III) score

3.2.3 Sample collection and 16SrRNA gene (rDNA) sequencing

Fecal samples were collected in tubes containing DNA stabilization buffer (CANVAX Biotech). For 16S rDNA sequencing, total microbial DNA was extracted using the QIAamp PowerFecal DNA Kit (Qiagen), according to the manufacturer's instructions. DNA concentration and purity were assessed, and samples were subsequently stored at −80 °C until further processing.

Library preparation for Illumina 16S metagenomic sequencing was performed starting from 12.5 ng of each DNA extract. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using universal primers described by Klindworth et al. [115], containing Illumina adapter

sequences. The resulting first-round PCR amplicons were purified with Agencourt AMPure XP beads (Beckman Coulter, Milan, Italy) before a second PCR step, which introduced dual indices and sequencing adapters using the Illumina Nextera XT Index Kit (Illumina Inc., San Diego, CA, USA) for library multiplexing. Following a second purification step, DNA concentrations were quantified with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific), diluted to 4 nM, and pooled for sequencing. Sequence data were generated as FASTQ files and deposited in the ArrayExpress repository under accession code E-MTAB-12486. Raw FASTQ reads were imported into QIIME2 (v2021.2 (44)), and Illumina primers were trimmed using the *q2-cutadapt* plugin in trim-paired mode [116]. Denoising of trimmed paired-end sequences was performed with the *q2-dada2* plugin [117]. Taxonomic assignment of amplicon sequence variants (ASVs) was carried out using the *q2-feature-classifier* plugin [118] with a pre-trained Naïve Bayes classifier based on the SILVA 138 database (99% operational taxonomic units, full-length sequences) [119]. The overall workflow, from sample collection to data analysis.

3.2.4 Statistical analysis

All statistical analyses were performed using RStudio (version 2023.12.1+402) running R (version 4.3.2). Data processing, statistical testing, and visualization were conducted on relative abundance data (normalized to 10 1 per sample) using the following R packages: *phyloseq, microbiome, vegan, ggplot2, dplyr, tidyr, FSA, scales, forcats, writexl*, and *broom*. Random seeds were fixed to ensure reproducibility.

To characterize differences in gut microbial diversity among clinical subgroups, a comprehensive multilevel statistical approach was employed.

Initially, alpha diversity was assessed using the Shannon and Simpson indices, which quantify within-sample microbial diversity by integrating both species richness and evenness.

These indices were calculated from rarefied abundance data to minimize sampling depth bias. Group-wise comparisons were performed using non-parametric tests (Wilcoxon rank-sum or Kruskal–Wallis, followed by Dunn's post hoc test where appropriate), in order to evaluate potential differences in microbial diversity across sex, H&Y stages, and the presence or absence of motor fluctuations.

Subsequently, beta diversity analyses were conducted to explore inter-sample community dissimilarity and compositional overlap among groups.

Bray–Curtis dissimilarity matrices were computed based on relative abundance data, and the resulting distance matrices were visualized through Principal Coordinates Analysis (PCoA). The statistical significance of group-wise clustering patterns was tested using Permutational Multivariate Analysis of Variance (PERMANOVA) [120] with 9,999 permutations, as implemented in the *vegan* R package (adonis function).
This multivariate test evaluates whether the centroids and dispersion of microbial communities differ significantly among predefined groups, thus providing a robust measure of compositional divergence.

Together, these analyses allowed us to determine whether microbial communities exhibited comparable internal diversity (α-diversity) or distinct overall community structures (β-diversity) when stratified by sex, disease severity (H&Y), and motor fluctuation status.
A false discovery rate (FDR) correction was applied to adjust for multiple testing where applicable, and a p-value < 0.05 was considered statistically significant.

To identify taxa showing significant compositional differences between PD patients with and without motor fluctuations, a differential abundance analysis was performed on the filtered dataset. Operational Taxonomic Units (OTUs) were first filtered to include only those with a relative abundance greater than 0.1% in at least 5% of all samples, ensuring that only prevalent and biologically meaningful taxa were retained for statistical testing.

This prevalence and abundance threshold was applied to reduce the influence of rare or spurious taxa that may introduce noise into the analysis.

Relative abundances were compared between the “No” (non-fluctuating) and “Yes” (fluctuating) groups using the non-parametric Wilcoxon rank-sum test, as implemented in R.

This test was chosen because microbiome relative abundance data are typically non-normally distributed and zero-inflated, making non-parametric methods more robust to deviations from Gaussian assumptions.

For each taxonomic rank (Phylum, Family, and Genus), taxa-level comparisons were computed, and p-values were subsequently adjusted for multiple testing using the Benjamini–Hochberg FDR correction. Adjusted q-values below 0.05 were considered statistically significant. In addition, effect sizes were estimated as $\log_2$ fold changes ($\log_2$FC) between the two groups. This analytical framework allowed the identification of specific microbial taxa that significantly differed between fluctuation phenotypes, highlighting taxonomic signatures potentially associated with dopaminergic instability and gut–brain axis dysfunction in PD.

To further explore potential relationships between microbial composition and clinical characteristics, a Spearman's rank correlation analysis was performed.

Correlations were computed between the 20 most abundant bacterial genera and selected clinical parameters, including age, disease duration, disease severity as measured by the H&Y stage, and motor symptom severity assessed by MDS-UPDRS III score.

Spearman's ρ coefficients were calculated to capture monotonic relationships without assuming data normality, and corresponding p-values were adjusted using the Benjamini–Hochberg FDR correction to control for multiple testing.

Finally, simple linear regression analyses were performed to evaluate the specific association between the relative abundance of *Faecalibacterium* and both the H&Y stage and MDS-UPDRS III scores.

Regression models were fitted using ordinary least squares (OLS), and model residuals were inspected to ensure homoscedasticity and absence of influential outliers.

These analyses allowed quantification of the directional relationship between *Faecalibacterium* abundance and clinical measures of disease progression and motor impairment.

3.3 Results

3.3.1 Microbiota diversity

Alpha diversity was assessed to evaluate within-sample microbial richness and evenness across different clinical and demographic variables. Diversity indices, including Shannon and Simpson, were calculated and compared between groups.

When stratified by sex, no significant differences in gut microbial diversity were observed between male and female participants as indicated by the Shannon ($p = 0.978$) or Simpson ($p = 0.778$, adjusted $p = 0.978$) indices. These findings suggest that sex did not exert a measurable influence on overall gut microbial diversity within this cohort (**Figure 12A**).

Similarly, alpha diversity measures did not differ significantly across H&Y stages (1–1.5, 2–2.5, and 3–4) with a slight tendency toward higher Shannon values in advanced one, suggesting that disease severity was not associated with changes in microbial richness or evenness (all adjusted $p > 0.26$; **Figure 12B**).

When patients were grouped according to the presence or absence of motor fluctuations, a trend toward higher alpha diversity was observed in fluctuating compared with non-fluctuating patients. However, these differences did not reach statistical significance after multiple-comparison adjustment (Shannon index: p = 0.029, adjusted p = 0.058; Simpson index: p = 0.077; **Figure 12C**). Overall, these results indicate that gut microbial alpha diversity remained relatively stable across sex, disease stage, and motor fluctuation status, suggesting that PD–related clinical features may have a limited effect on within-sample microbial diversity in this population.

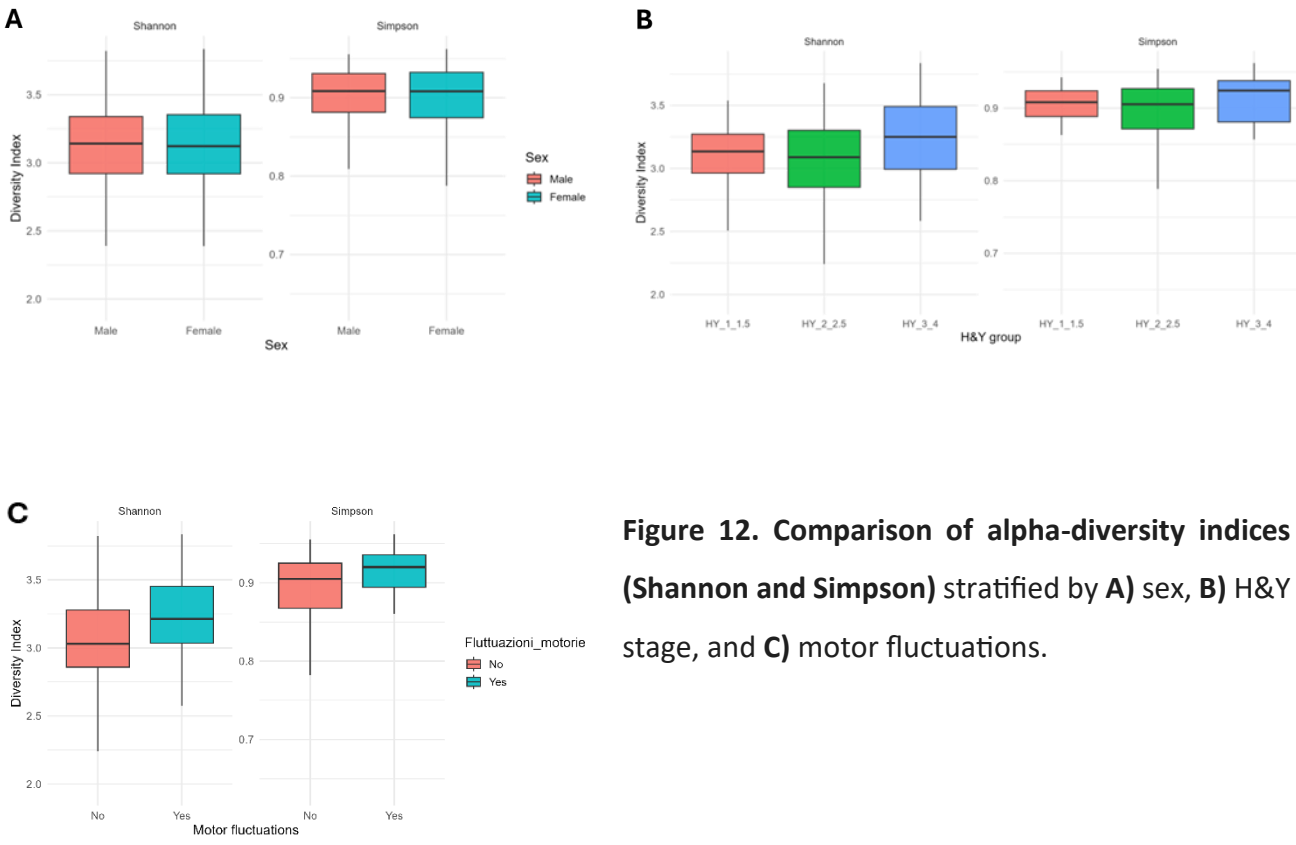

Figure 12. Comparison of alpha-diversity indices (Shannon and Simpson) stratified by **A)** sex, **B)** H&Y stage, and **C)** motor fluctuations.

3.3.2 Beta diversity

Beta diversity was evaluated to assess differences in the overall gut microbial community structure among study subgroups. PCoA based on Bray–Curtis dissimilarity was used to visualize inter-individual dissimilarities, while PERMANOVA was applied to test statistical significance across groups.

When comparing male and female participants, PCoA plots showed a substantial overlap of samples, with no evidence of distinct clustering by sex (**Figure 13A**). Consistently, PERMANOVA revealed no

significant differences in beta diversity between males and females (R^2 = 0.0069, F = 0.747, p = 0.683), indicating that sex did not significantly contribute to the observed variability in microbial composition.

To explore the potential influence of disease severity, patients were next stratified according to H&Y stage categories (1–1.5, 2–2.5, and 3–4). The resulting PCoA ordination displayed partial overlap among groups, with no clear separation along the main coordinate axes (**Figure 13B**). PERMANOVA analysis did not detect statistically significant compositional differences across stages (R^2 = 0.0276, F = 1.503, p = 0.080), suggesting that disease progression was not associated with substantial changes in the global gut microbial structure.

Conversely, when comparing PD patients with and without motor fluctuations, PCoA plots suggested a modest but noticeable separation between the two groups (**Figure 13C**). This observation was confirmed by PERMANOVA, which identified a statistically significant difference in microbial composition between fluctuating and non-fluctuating patients (R^2 = 0.0298, F = 3.282, p = 0.003*). Although the proportion of variance explained by fluctuation status was relatively small (~3%), the finding indicates that the presence of motor fluctuations is associated with detectable shifts in gut microbial community composition.

Overall, these analyses demonstrate that beta diversity was largely unaffected by sex or disease stage, whereas motor fluctuation status emerged as a significant factor influencing gut microbial composition. This suggests that clinical manifestations related to disease variability, rather than static demographic or severity measures, may have a more pronounced relationship with microbiota structure in PD.

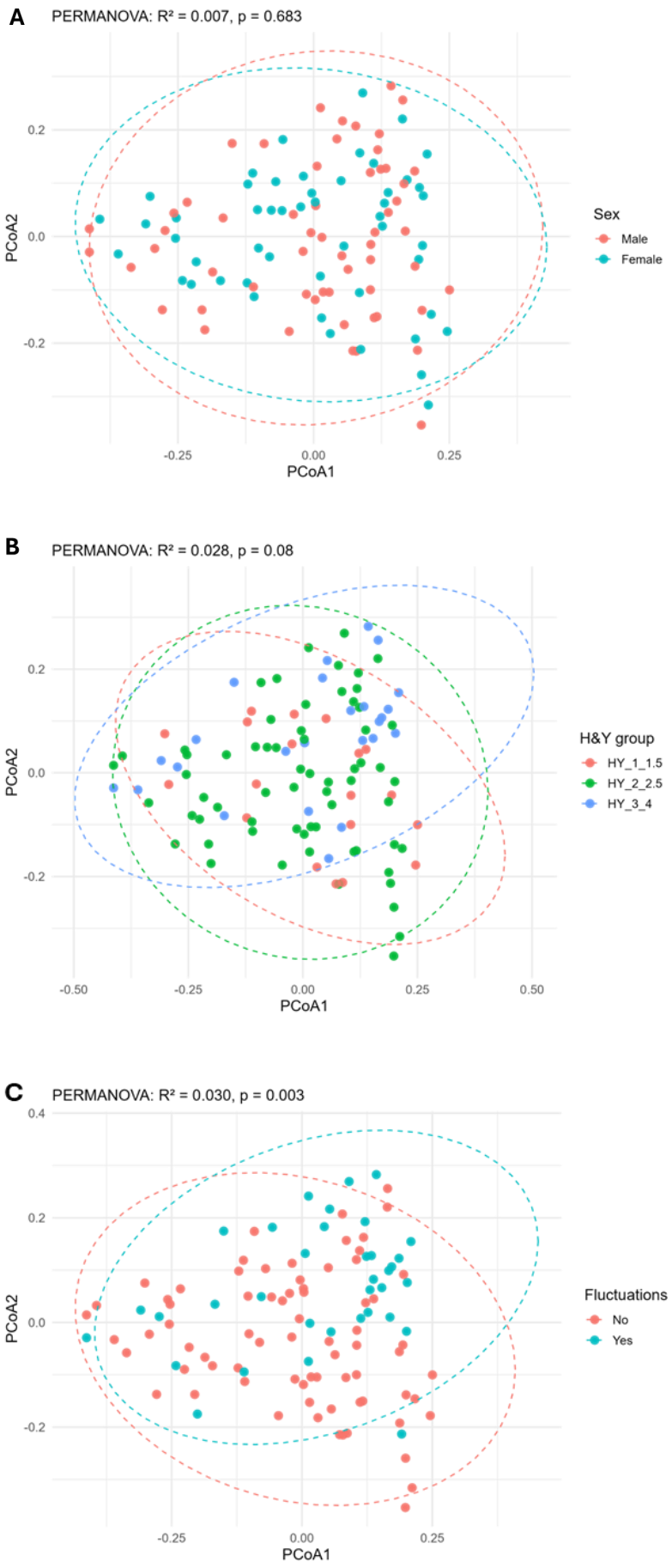

Figure 13. Principal Coordinates Analysis (PCoA) of beta diversity showing sample distribution across groups: A) sex, **B)** H&Y stage, and **C)** motor fluctuations. Ellipses represent 95% confidence intervals for each group.

3.3.3 Differential abundance of microbial taxa according to motor fluctuation status

Following filtering at >0.1% relative abundance in ≥5% of samples, 119 genera were retained for differential abundance analysis. The GM of PD patients was predominantly composed of Firmicutes and Bacteroidetes, with smaller contributions from Proteobacteria and Actinobacteria (**Figure 14A**). These major phyla accounted for the vast majority of the bacterial community across all subjects, with no substantial differences observed between patients with and without motor fluctuations.

At lower taxonomic levels, more pronounced group-specific variations were identified (**Figure 14C, Figure 15**). Several genera exhibited significant differential abundance between fluctuating and non-fluctuating PD patients. Specifically, *Lachnoclostridium* (p-adj = 0.018) and unclassified *Lachnospiraceae* (p-adj = 0.022) were notably depleted in the fluctuating group. These taxa are well-recognized producers of SCFAs such as butyrate, which play a crucial role in maintaining intestinal barrier integrity and anti-inflammatory homeostasis. Their reduction could therefore suggest impaired SCFA-mediated regulatory functions in patients experiencing motor fluctuations.

Conversely, fluctuating patients showed higher relative abundances of unclassified *Eubacteriaceae* (p-adj = 0.018), *Alistipes* (p-adj = 0.022), and *Collinsella* (p-adj = 0.022). These taxa have been previously associated with altered mucin degradation, bile acid metabolism, and pro-inflammatory activity, processes that may influence intestinal permeability and local immune activation. Additional genera enriched in the fluctuating group included unclassified *Bacilli* (p-adj = 0.022), unclassified *Clostridia* (p-adj = 0.033), *Desulfovibrio* (p-adj = 0.044), and *Lactobacillus* (p-adj = 0.044). The presence of these bacteria may reflect a shift toward microbial communities adapted to oxidative or metabolic stress conditions, consistent with previous evidence linking intestinal dysbiosis to disease progression and treatment response variability in PD.

In addition, the reduction of beneficial genera such as *Roseburia* (p-adj = 0.029) and *Faecalibacterium* (p-adj = 0.058) further supports the notion of a less butyrogenic microbiota in fluctuating patients. Although differences in other genera, including *Bilophila*, *Butyrivibrio*, and *Fusicatenibacter*, did not reach statistical significance after adjustment (p-adj = 0.058), they followed a consistent downward trend, reinforcing this overall pattern.

Taken together, the observed taxonomic shifts suggest that patients with motor fluctuations harbor a GM characterized by decreased SCFA-producing capacity and increased representation of taxa involved in mucin and bile acid metabolism. This compositional profile may contribute to a pro-inflammatory intestinal milieu and altered gut–brain metabolic signaling, potentially influencing dopaminergic drug metabolism and the fluctuating clinical course of PD.

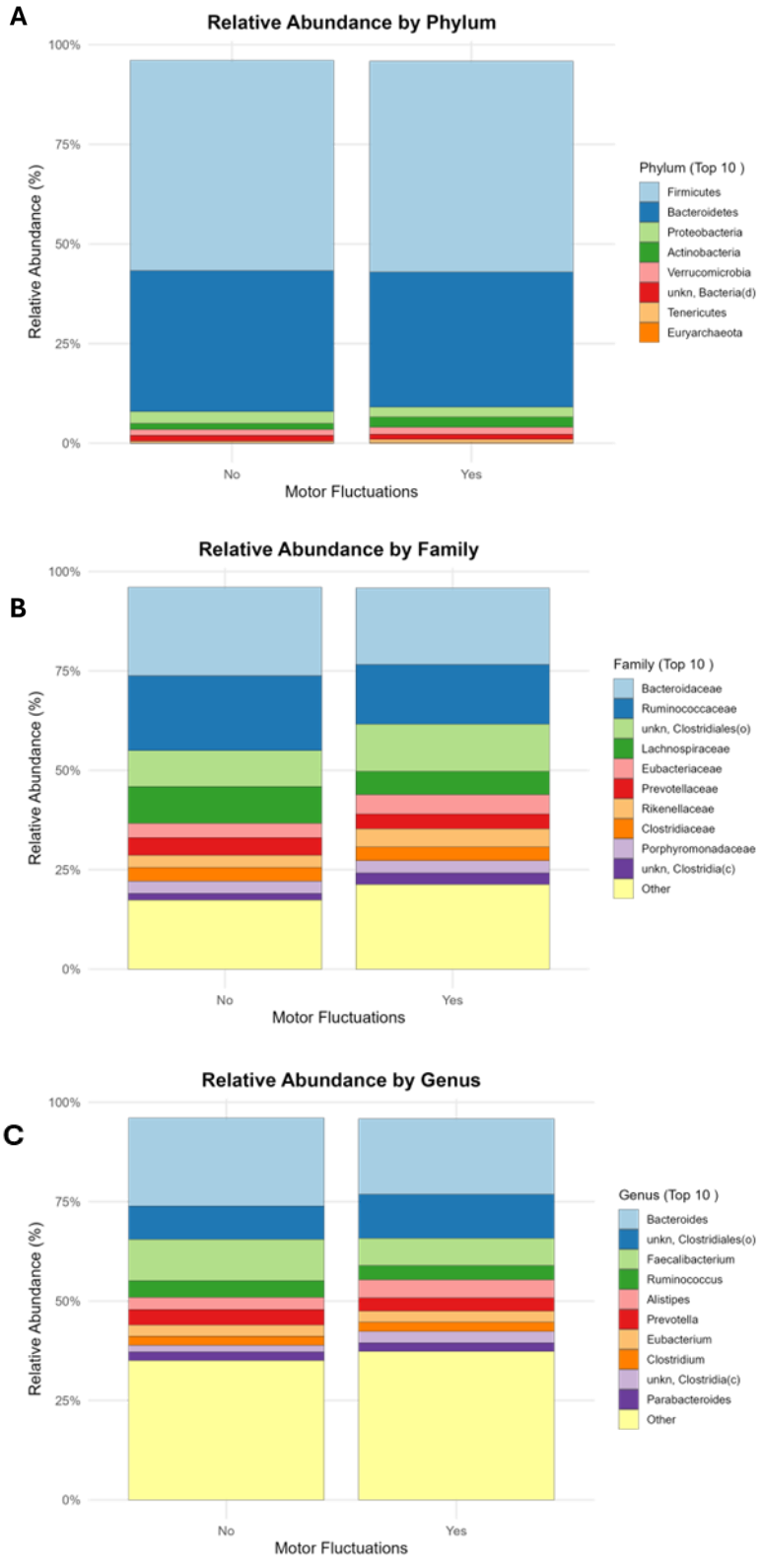

Figure 14. The 10 most common bacterial taxa in patients with and without fluctuations. A) At Phylum level; **B**) at Family level; **C**) at Genus level.

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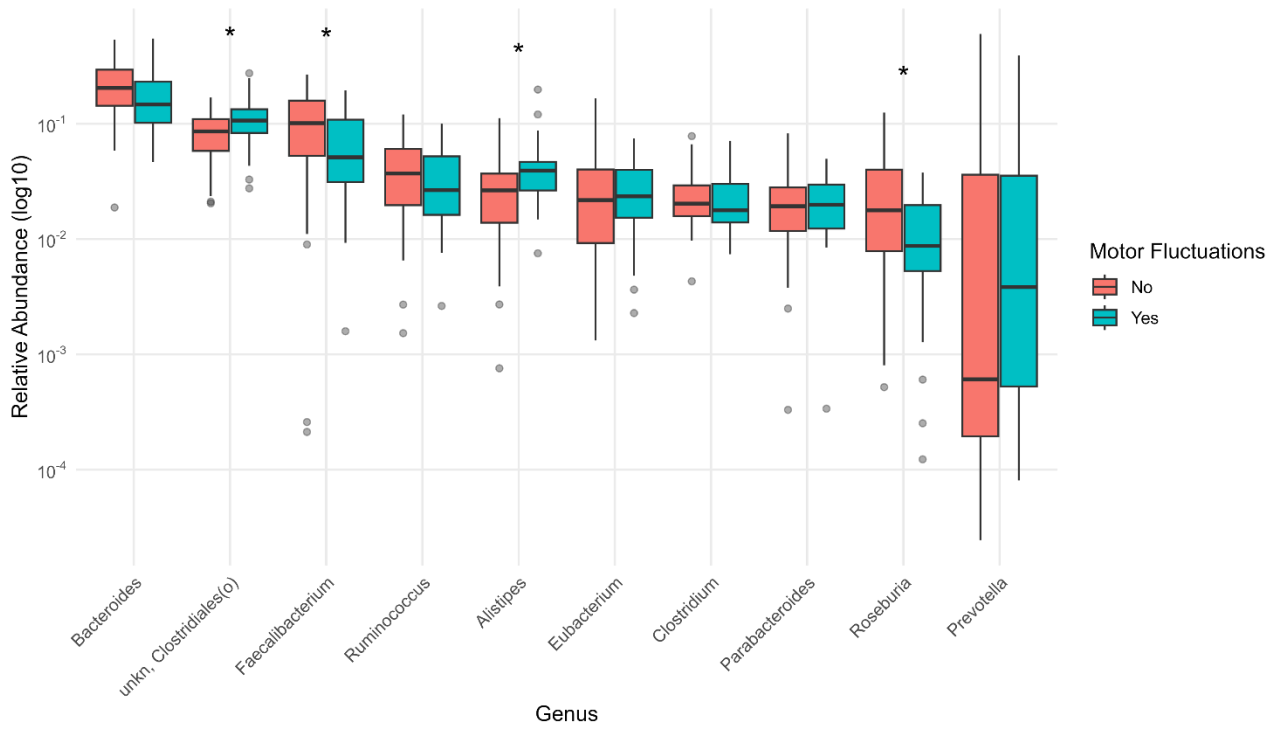


Figure 15. Genus-level microbial composition differences between PD patients with and without motor fluctuations

Relative abundances are displayed on a logarithmic scale. Statistically significant differences were determined using the Wilcoxon rank-sum test. * (*p-value* < 0.05).

3.3.4 Associations between microbial genera and clinical variables

Spearman correlation analysis (Benjamini–Hochberg adjusted) was performed between the 20 most abundant bacterial genera and key clinical variables, including age, disease duration, H&Y stage, and MDS-UPDRS III motor score (**Figure 16**).

Distinct correlation patterns emerged, highlighting specific microbial signatures associated with PD severity and progression.

Negative correlations were observed between *Faecalibacterium*, *Roseburia*, *Lachnoclostridium*, and unclassified *Lachnospiraceae*(f) with both disease duration and MDS-UPDRS III scores. These associations (all p-adj < 0.01) indicate that the depletion of these SCFA–producing genera parallels longer disease duration and worse motor function, suggesting a link between reduced butyrate-producing capacity and neurodegenerative progression. *Faecalibacterium* also exhibited a significant negative correlation with H&Y stage (ρ = –0.36, p-adj = 0.0039), reinforcing its association with advancing disease severity.

Conversely, *Alistipes* and *Parabacteroides* displayed positive correlations with both disease duration and MDS-UPDRS III, although these did not survive multiple-comparison adjustment (p-adj > 0.05). These genera have previously been linked to mucin degradation and pro-inflammatory metabolic activity, suggesting their increase may accompany disease-related intestinal dysfunction.

Unclassified *Eubacteriaceae*(f) and *Clostridia*(c) showed weak positive correlations with Disease Duration and MDS-UPDRS III (ρ = 0.17–0.19), consistent with a potential enrichment of pro-inflammatory taxa in more advanced disease stages, though these associations were not statistically significant after adjustment. No significant correlations were observed between any genus and LEDD, and only weak associations with age were detected, suggesting that medication dosage and chronological aging exert a minor influence on microbial composition compared with disease-related factors.

Overall, the correlation heatmap revealed two distinct bacterial clusters. The first cluster—comprising *Faecalibacterium*, *Roseburia*, *Lachnoclostridium*, and *Lachnospiraceae*(f)—represents a "protective SCFA-producing" group, characterized by negative correlations with disease severity and duration, consistent with their role in SCFA production and gut homeostasis. In contrast, taxa such as *Alistipes*, *Parabacteroides*, and unclassified *Eubacteriaceae*(f) formed a "pro-inflammatory dysbiosis-associated" group, positively associated with disease parameters and potentially contributing to mucosal inflammation and metabolic imbalance.

This bidirectional pattern is clearly visible in the hierarchical clustering of the lateral dendrograms (**Figure 16**), which group together genera exhibiting similar correlation profiles, emphasizing a functional dichotomy between SCFA-producing and pro-inflammatory microbial taxa in PD.

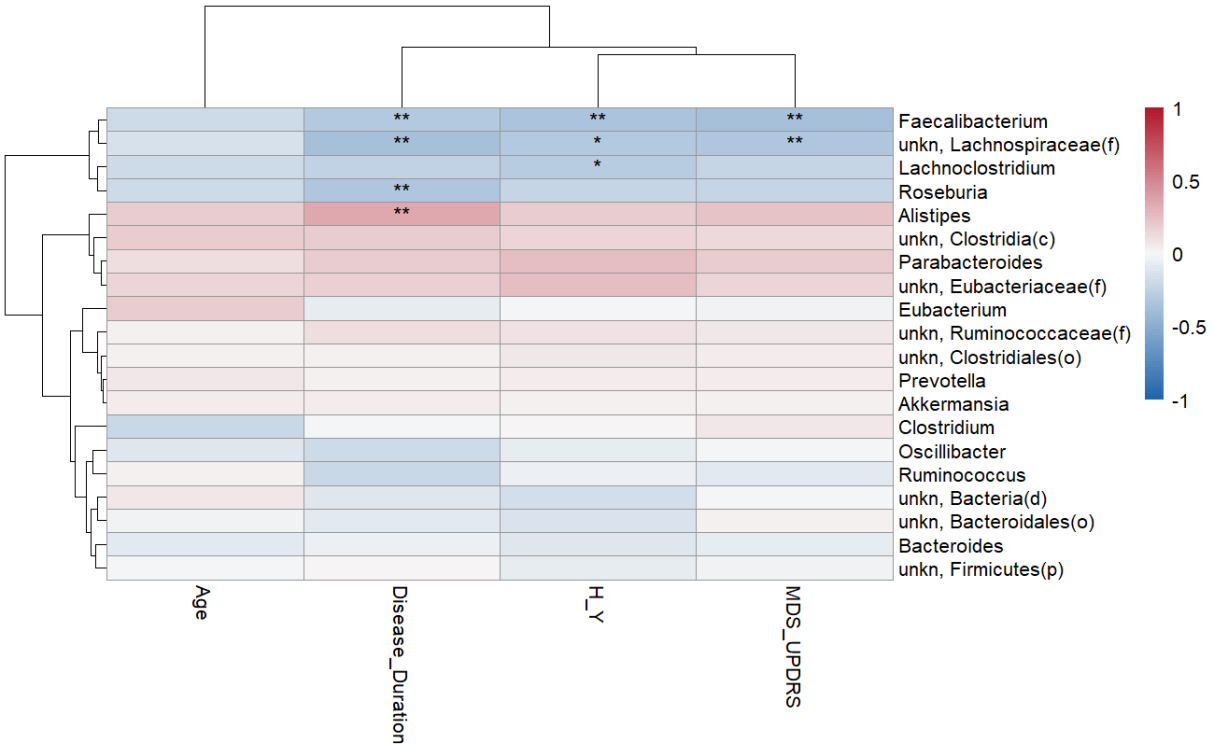


Figure 16. Heatmap showing Spearman correlations between most abundant bacterial genera and clinical variables

Colors indicate correlation strength (red = positive; blue = negative).

Linear regression analyses were conducted to explore the association between *Faecalibacterium* relative abundance and clinical markers of PD severity.

A significant negative correlation was found between *Faecalibacterium* abundance and H&Y stage ($\beta = -0.0293$, $R^2 = 1.00$, $p = 0.00117$; **Figure 17A**), indicating a marked reduction of this short-chain fatty acid (SCFA)–producing genus with advancing disease stage.

Similarly, *Faecalibacterium* abundance was inversely associated with MDS-UPDRS III motor scores ($\beta = -0.00198$, $R^2 = 1.00$, $p = 0.00019$; **Figure 17B**), suggesting that lower levels of this beneficial taxon are linked to more severe motor dysfunction.

Together, these findings highlight a consistent, severity-dependent depletion of *Faecalibacterium* in PD, reinforcing its potential as a microbial biomarker of disease progression and motor impairment.

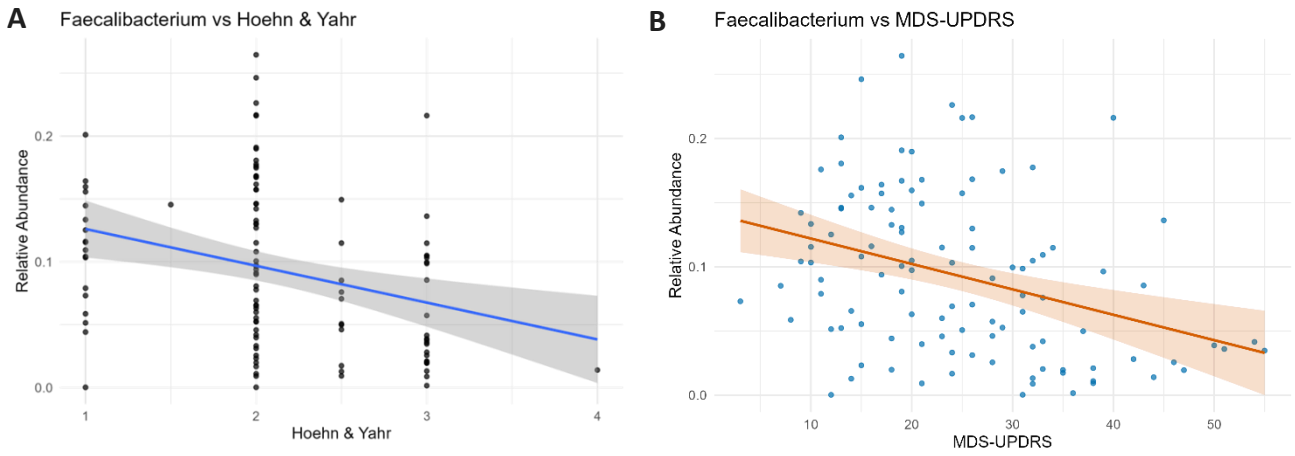


Figure 17. Linear regression analyses showed a significant negative correlation between *Faecabilibacterium* and **A)** Hoehn & Yahr (H&Y) stage and **B)** MDS-UPDRS III motor scores

3.4 Discussion

This study explored the relationship between GM composition and clinical features in PD, with particular attention to the presence of motor fluctuations, disease severity, and motor symptom burden. Through an integrated analysis of microbial diversity, differential abundance, and clinical–microbial correlations, we observed that while global microbial diversity remained largely preserved, specific taxonomic and functional alterations were associated with the progression and fluctuating course of the disease [121,122].

The analysis of α-diversity, reflecting within-sample microbial richness and evenness, revealed no significant differences across sex or disease severity, and only a non-significant trend toward higher diversity in patients with motor fluctuations. These findings are consistent with several previous studies reporting that the total richness of the intestinal microbiota in PD often remains stable or may even increase with the progression of the disease [123].

In contrast, compositional and functional imbalances are more relevant to disease pathophysiology. The slight, non-significant increase in Shannon diversity observed in the most advanced H&Y stages may reflect compensatory microbial remodeling rather than a true enrichment in beneficial taxa. Overall, these results suggest that disease progression in PD is not characterized by a generalized microbial impoverishment, but by selective compositional shifts involving key metabolic and inflammatory pathways.

In contrast, beta diversity analysis highlighted that the overall structure of the gut microbial community was significantly influenced by the presence of motor fluctuations. Patients experiencing fluctuations displayed a distinct clustering pattern compared to non-fluctuating subjects, indicating detectable differences in microbial community composition. Although the proportion of variance explained by fluctuation status was modest, this finding suggests that dynamic aspects of PD, such as dopaminergic response instability, may be linked to specific alterations in gut microbial networks [124]. This observation aligns with the growing evidence that gut microbial metabolism can modulate dopaminergic signaling through mechanisms involving the production or degradation of levodopa, modulation of intestinal permeability, and immune–neural crosstalk along the gut–brain axis.

Differential abundance analysis revealed a complex microbial signature distinguishing fluctuating from non-fluctuating PD patients. Fluctuating patients exhibited a marked reduction in butyrate-producing genera such as *Lachnoclostridium*, *Roseburia*, *Faecalibacterium*, and unclassified *Lachnospiraceae*. These taxa are key producers of SCFAs, particularly butyrate, which serves as an essential energy source for colonocytes and exerts potent anti-inflammatory and neuroprotective effects [125,126]. Their depletion may indicate a reduced capacity of the gut ecosystem to maintain intestinal barrier integrity and suppress chronic inflammation, both of which are increasingly recognized as relevant contributors to PD pathogenesis. Conversely, an increased abundance of *Alistipes*, *Collinsella*, *Desulfovibrio*, and unclassified *Eubacteriaceae* and *Clostridia* was observed in fluctuating patients. These bacteria have been associated with mucin degradation, bile acid dysmetabolism, and LPS-mediated pro-inflammatory responses, which may amplify intestinal permeability and systemic inflammation. Such processes could facilitate peripheral immune activation and contribute to microglial sensitization, thereby influencing neurodegenerative progression [127].

The functional implications of these microbial shifts are particularly relevant in the context of motor fluctuations. The enrichment of *Desulfovibrio* and *Alistipes*—genera capable of producing hydrogen sulfide and other potentially neurotoxic metabolites—may contribute to local oxidative stress and impaired absorption or metabolism of dopaminergic therapies. On the other hand, the reduction of SCFA producers may limit the availability of anti-inflammatory metabolites such as butyrate and propionate, which are known to regulate dopaminergic neuron survival, BBB permeability, and neuroimmune homeostasis. Therefore, the imbalance observed between beneficial SCFA-producing

and potentially deleterious taxa may represent a microbial correlate of dopaminergic instability and motor fluctuation occurrence.

Correlation and regression analyses further reinforced these observations, linking specific bacterial taxa to clinical indices of disease progression. Negative correlations between *Faecalibacterium*, *Roseburia*, *Lachnoclostridium*, and unclassified *Lachnospiraceae* with both disease duration and MDS-UPDRS III scores indicate that depletion of these beneficial taxa parallels worsening motor performance and longer disease course [121]. In contrast, positive correlations between unclassified *Eubacteriaceae* and *Clostridia* with clinical severity suggest that their proliferation accompanies neurodegenerative advancement and motor instability. *Alistipes* exhibited mixed associations with age and disease severity, while *Akkermansia* remained relatively stable, reflecting preserved mucin-degrading capacity independent of clinical status.

Linear regression analyses focusing on *Faecalibacterium* abundance provided further confirmation of these trends. The significant inverse associations between *Faecalibacterium* and both H&Y stage and MDS-UPDRS III scores underscore the consistent depletion of this SCFA-producing genus with increasing disease severity. Given the well-established anti-inflammatory and neuroprotective roles of *Faecalibacterium prausnitzii*, its reduction may reflect a loss of microbial resilience contributing to intestinal and central neuroinflammation. Although the relationship between microbial diversity and disease severity was not linear, the decline in specific beneficial taxa highlights the selective nature of PD-related dysbiosis, involving targeted disruptions in microbial function rather than a global collapse of community diversity [128].

Taken together, the findings from this study suggest that the GM in PD is characterized by selective functional and compositional alterations that become more pronounced in the presence of motor fluctuations. Rather than generalized dysbiosis, PD appears to involve a progressive imbalance between anti-inflammatory, butyrate-producing bacteria and pro-inflammatory, mucin-degrading taxa. This imbalance may contribute to impaired gut barrier function, altered dopamine metabolism, and chronic neuroinflammation, all of which are potential mediators of disease progression and motor fluctuation development.

These results not only reinforce the link between GM and PD pathophysiology but also raise the possibility that targeting microbial function—through dietary modulation, prebiotic or probiotic supplementation, or microbiota transplantation—could represent a complementary therapeutic approach to stabilize motor function and slow disease progression.

Additionally, it would be beneficial to further investigate the presence of specific bacteria, such as *Enterococcus faecalis*. This bacterium produces the enzyme decarboxylase, which metabolizes L-dopa. This metabolism may reduce the effectiveness of therapy in patients with advanced PD who exhibit more severe symptoms [109].

Future longitudinal and mechanistic studies integrating metagenomic and metabolomic data will be essential to determine whether these microbial alterations precede clinical changes or arise as secondary phenomena, and to identify specific microbial metabolites that may influence dopaminergic homeostasis and treatment responsiveness in PD.

4. Changes in gut microbiota composition after 12 weeks of a home-based lifestyle intervention in breast cancer survivors during the COVID-19 lockdown

4.1 Introduction

BC is the most common malignancy in women worldwide and continues to be a cause of death despite screening, early diagnosis, and personalized treatments. Incidence rates of BC have been increasing by about 0.5% per year, and the expected worldwide incidence of BC by 2050 is estimated to be 3.2 million new cases per year [129,130]. In Italy, there are around 55000 newly diagnosed cases of cancer each year, with 13000 resulting in death. These cases account for 14.6% of new cancer diagnoses [131,132]. From an etiological point of view, the onset of BC can be influenced by genetics or epigenetics (e.g., Micro-RNA miR-21, which controls various genes involved in tumor progression) and age (young age is a negative risk factor for BC). It can lead to advanced stages or lower survival rates, reproductive factors (the breast cells differentiation process during pregnancy could be protective), and lifestyle (e.g., less physical activity) [129,130]. This worrying data reflects the need for rigorous prevention and treatment strategies and further studies to understand all possible factors influencing the development of BC and its recurrence.

The GM is a newly emerging field of study as an additional environmental risk factor in a patient with BC, especially associated with metabolic dysfunctions. Recent evidence has described the role of GM dysbiosis in the development of BC via estrogen-dependent mechanisms linking the production of microbial-derived metabolites, adaptive immune response, and estrogen metabolism [133]. Moreover, microbial metabolites have been demonstrated to express anticancer properties, including lithocholic acid, butyrate, and cadaverine [134,135]. Novel approaches targeting the GM have been proposed to preserve or restore normobiosis in the prevention and treatment of BC [134]. The GM is influenced by several factors: some are unmodifiable (e.g., age, genetics, sex), and others are modifiable (e.g., lack of a regular diet and physical activity) [136]. In particular, diet is a key component of the relationship between humans and their microbial residents and plays an important role in modulating its composition and diversity, affecting human health through this interaction [137]. Changes in the host's eating habits are reflected in the bacterial metabolism and induce the proliferation of the species most suitable for the use of the nutrients consumed [138]. A western type diet, rich in fat, salt, meat, refined flour, and sugar, can induce a state of dysbiosis,

which is a change in the structural and functional balance of the gut microbial population often associated with obesity, diabetes and chronic inflammation [139]. It has been recognized that 35% of all cancers are associated with dietary intake, including 50% of breast carcinomas [140], and it is likely that microbiota can be involved in carcinogenesis. On the other hand, correct dietary habits can positively influence intestinal microbiota, restore gut eubiosis, and hence improve overall host health.

The Mediterranean Diet (MD) is recognized by UNESCO as a cultural heritage of humanity. It is considered one of the healthiest nutritional patterns because it prefers raw foods to processed ones, and it provides a high consumption of vegetables, fruits, whole grains, unsaturated fats such as olive oil, fish, eggs, nuts, legumes, and poultry, reducing red meat and sugars [141]. Along with protection against diabetes and metabolic diseases, a reduction in BC has also been shown [142]. It has been demonstrated that MD improves the microbiota composition compared to other diets [143]. Moreover, Pellegrini et al. [144] demonstrated that MD, in addition to probiotics, can positively affect GM composition and that these effects translate into the improvement of metabolic and anthropometric parameters.

Low saturated and high ω-3 fatty acids intake has been linked to anti-inflammatory signaling, which is also mediated by microbiota [145,146]. High amounts of fibers and polyphenols that exert a prebiotic action on specific strains lead to increased SCFA, lowering cancer risk and cardio-metabolic diseases [147,148]. Fermentable fibers allow bacteria to produce butyrate, acetate, and propionate [149] that regulate immunity and metabolism via microbiota [150]. The protection from cancer seems to be improved by increased *Bifidobacterium* genera [151] and decreased *Fusobacterium nucleatum* [143]. Furthermore, adherence to the MD increases a-diversity, which expresses the number of species present in the microbiota and positively correlates with overall health [152].

In both animal and human investigations, physical exercise was associated with favorable modifications in the GM's diversity, richness, and composition [153]. Positive associations have been shown in humans between butyrate-producing bacteria, alpha diversity, and cardiorespiratory fitness [154], as well as between athletes and sedentary controls in terms of SCFA concentrations and higher turnover of carbohydrates and proteins. When fecal samples from nineteen people belonging to three cohorts (adults elite, youth elite, and youth non-elite athletes) were analyzed, it was found that top athletes had higher microbial diversity, a distinct taxonomic and functional composition, and a substantial correlation with athletic performance [155]. Exercise alone does not account for changes in the microbiota composition; these changes also have to do with nutritional

consumption, which research has shown to be altered by exercise itself [156]. In a study by Donati Zeppa et al. [157], nine weeks of high-intensity exercise caused a shift in the gut microbial population towards a healthier microbiome in healthy male college students. Moreover, changes in the GM composition were correlated with dietary, body composition, and performance variables. Cheng et al. [158] recently demonstrated that a combined 8.6-month aerobic exercise and low-carbohydrate dietary intervention increased microbiome diversity and stability in patients with nonalcoholic fatty liver disease. Further, Furber et al. [159] reported a significant association between microbial stability following a dietary intervention and athletic performance in a cohort of highly trained athletes.

Several studies have been carried out on the role of microbiota in human health, and particular attention should be paid to the frail population. In this regard, BC survivors represent a population in which the combined influence of cancer treatment and advancing age often coincides with several physiological changes harmful to cardio-metabolic health, including increased adiposity [160], dysbiosis [161], and reduced cardiorespiratory fitness [162]. When BC survivors complete primary treatments (i.e., chemotherapy and radiotherapy), women face several challenges in the long term, and some of them continue with hormone therapy for 5 to 10 years. Side effects of treatments include significant changes in anxiety, fatigue, and sleep dysfunction [163], as well as changes in the metabolic profile (36). Thus, interventions based on diet and physical activity have the potential to reduce comorbidity and prevent BC recurrences [164,165]. An increasing need for such lifestyle interventions was highlighted during the pandemic emergency, which further increased the health risks for people with a poor lifestyle [166,167].

We previously reported that a 12-week home-based lifestyle intervention focused on MD and an aerobic exercise training program performed during the COVID-19 home confinement significantly ameliorated cardiometabolic parameters in BC survivors [168]. Here we report the gut microbial composition before and after the lifestyle intervention in twenty BC survivors. The present study explores the effect of lifestyle (MD and aerobic exercise) in modulating the gut microbial composition in twenty BC survivors during the first wave of the COVID-19 lockdown.

4.2 Methods

4.2.1 Study design and population

The MoviS clinical trial (protocol no. NCT04818359) was designed as an open-label randomized controlled trial with two parallel groups (1:1 randomization ratio with the control arm). However, due to the imposed COVID-19 pandemic restrictions, after the approval of the institutional ethics committee, the study protocol was amended (protocol no. 29/20, 22/04/2020). As reported by Natalucci et al. [168], the forced changes in the study protocol made the difference in cardiometabolic parameters between the intervention and control arms negligible, providing similar adaptations between groups. Therefore, due to the lack of meaningful differences between the two interventions, the results of the two groups were combined. Informed consent was obtained from all individual participants included in the study, and the research was performed in accordance with the Declaration of Helsinki. The women included in this study were recruited at the "Santa Maria della Misericordia" Hospital of Urbino (Italy), as they were eligible for the inclusion and exclusion criteria reported in https://clinicaltrials.gov/ct2/show/NCT04818359. A sample size was considered (n=20), according to a previously published paper [163].

4.2.2 Lifestyle intervention

Following surgery and primary therapies (chemotherapy and/or radiotherapy), eligible participants participated in a lifestyle intervention. As previously described, participants (intervention and control arms) received 12-week lifestyle (nutrition and exercise) educational counseling and program [168]. All participants received nutritional advice based on MD during the intervention phase, while only the intervention arm participated in a 12-week exercise training program (2 on-site and 1 remotely supervised aerobic session per week) with progressive increases in exercise intensity (from 40% to 70% of heart rate reserve) and duration (from 20 to 60 min).

Although different methods can be used to prescribe exercise intensity [169,170], heart rate (HR) reserve was used in the current study to account for physiological adjustments that occur during prolonged aerobic exercise (e.g., cardiovascular drift) [171]. The recommended quality (exercise intensity) and quantity (exercise volume) of aerobic exercise for participants were attained and exceeded gradually by increasing exercise intensity and duration [172]. However, as a result of COVID-19 pandemic restrictions put in place, from the 4th week, the type of supervision was

adapted to exclusively remotely supervised aerobic exercise (3 sessions per week). Weekly phone calls from the exercise specialist, who provided the weekly exercise prescription and tailored feedback based on the training logs, were used for supervision.

Both remotely and on-site supervised training sessions consisted of aerobic exercise (i.e., walking, running, or cycling). On-site aerobic exercise sessions (for the first 4 weeks) were performed using a treadmill or stationary bikes. While remotely supervised sessions were performed using treadmill or stationary bikes, if the participant had them available at home or through other outdoor exercises (e.g., walking without the aid of tools) according to the possibilities and preferences of the participants. Regardless of the exercise modality, the sessions were performed at individualized exercise intensities (e.g., walking speed and grade or cycling wattage), allowing each participant to reach and maintain the prescribed target HR during the training sessions.

4.2.3 Sample collection and 16SrRNA gene (rDNA) sequencing

Fecal samples were collected at baseline and after 12 weeks in tubes filled with DNA stabilization buffer (CANVAX Biotech). For 16S rDNA sequencing, the total microbial DNA was extracted using the QIAamp PowerFecal DNA Kit (Qiagen) following the manufacturer's protocol. After assessing DNA concentration and purity, samples were stored at -80° until processing. Library Preparation for the Illumina 16S Metagenomic Sequencing protocol was implemented starting from 12.5 ng of each DNA extract which was subjected to V3–V4 hypervariable regions of the bacterial 16S rDNA amplification using the universal primers previously published by Klindworth et al. [115] with Illumina adapters. Agencourt AMPure XP beads (Beckman Coulter, Milan, Italy) were utilized for the first PCR amplicons purification before the second round of PCR aimed to barcode the libraries using the Illumina dual-index system (Nextera XT Index Kit, Illumina Inc., San Diego, CA, USA) necessary for multiplexing. After the second purification step, the Qubit dsDNA BR Kit assay was employed to quantify the eluted DNA, which was then diluted to 4nM and pooled. Sequence data generated as FASTQ files, deposited in the Arrayexpress repository under accession code E-MTAB-12486. FASTQ raw sequencing data were imported into QIIME2 v.2021.244, and Illumina primers were removed using the q2-cutadapt plugin in trim-paired mode [116]. Denoising of trimmed sequences was performed in paired-end mode using the q2-dada2 plugin [117]. The q2-feature-classifier plugin was used to perform the taxonomy assignment of the amplicon sequence variants (ASVs) [118] based on the pre-trained Naïve Bayes classifier SILVA 138 99% operational taxonomic units (OTUs) full-

length sequence dataset [119]. The workflow from sample collection to data analysis is reported in **Figure 18**.

4.2.4 Statistical analysis

Data are reported as median and quartiles (Q1-Q3). The microbial alpha diversity indexes (OTUs number and Shannon's Effective Number of Species (ENS)), which describe the richness of the microbial community [173], were calculated with the *diversity* function of the *vegan* R package, in pre- and post-intervention, and compared by a Wilcoxon signed-rank test for paired data. The choice for the use of Shannon's effective number is that it describes the taxa to be considered important in a sample, and it is calculated by the exponential of the Shannon-Wiener index, which considers the differences in the abundance of each species. For further analysis, data were filtered for a relative abundance of 0.1% in both pre- and post-training conditions. A PERMANOVA for repeated measures on a Bray-Curtis distance matrix was applied to test pre-post differences in the relative abundances at different taxonomic levels; *post-hoc* comparisons were conducted with a Wilcoxon test, and a FDR with Benjamini-Hochberg correction was applied to account for multiple comparisons. Effect sizes (r) were then calculated with the *rstatix* and *coin* R packages. To assess pre-post changes in global genera abundances, a non-metric dimensional scaling (nMDS) with Bray-Curtis distance was used. A multivariate analysis of variance for repeated measures (RM-MANOVA) was applied to test pre-post changes of physical and hematological variables. Lastly, a correlation plot was built to explore correlations between pre-post variations of genera abundances and variations of physical and hematological parameters; for graphical reasons, only significant correlations were reported ($r_{0.05,20} \geq 0.378$). Statistical analyses were conducted using R Studio 4.1; the alpha value was set at the standard level of 0.05.

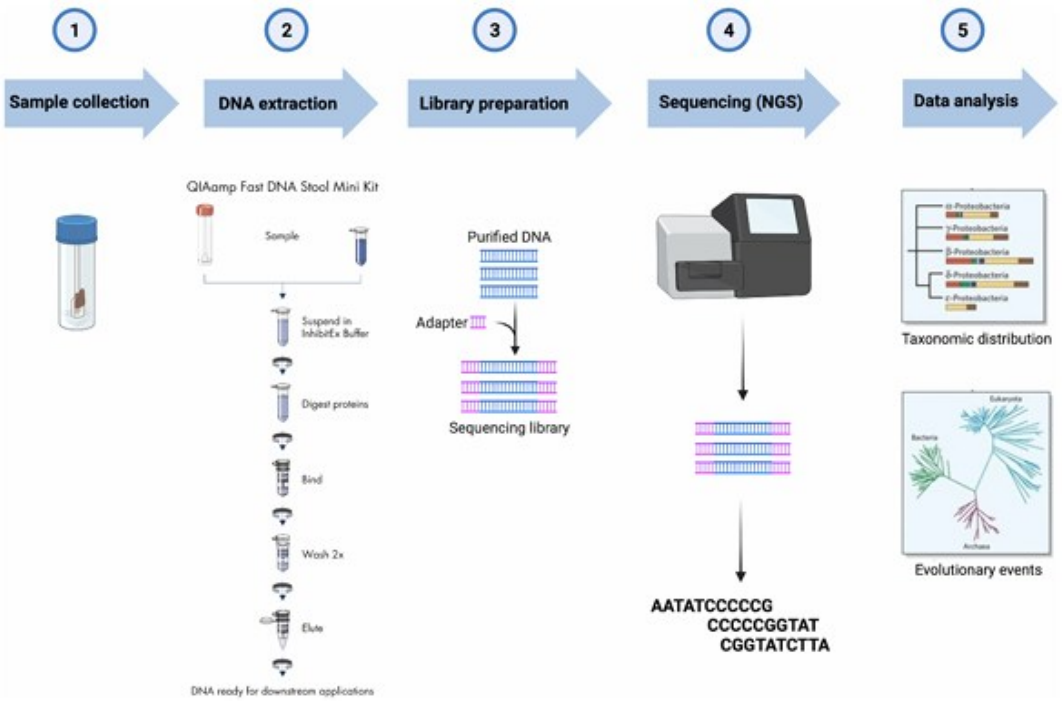


Figure 18. Workflow of the 16S rRNA gene analysis of fecal samples collected

4.3 Results

4.3.1 Sample characteristics

The analysis included twenty women who were originally enrolled in the main trial.

Baseline sample characteristics are described in **Table 6**. The mean age of the sample was 51.8 ± 7.8 years old, and the time since diagnosis was 10.2 ± 3.1 months.

Variations in cardiorespiratory fitness, body composition, blood biomarkers, and MD adherence are reported in **Table 7**. Significant changes in the post-intervention measures were detected for maximal oxygen uptake ($\dot{V}O_{2max}$), fasting glucose, testosterone concentration, Homeostatic Model Assessment (HOMA), triglycerides, LDL, and adherence to MD (MeDiet Score by DianaWeb, as reported in Natalucci et al. [168]).

	n	%
Disease stage		
0	4	20%
I	10	50%
II	6	30%
III	-	-
Menopausal Status at diagnosis		
Pre-menopausal	3	15%
Post-menopausal	17	85%
Surgery type		
Mastectomy	3	15%
Quadrantectomy	16	80%
Lumpectomy	1	5%
Treatment in addition to surgery		
Radiation	7	35%
Chemotherapy	16	80%
Radiation + Chemotherapy	7	35%
None	4	20%
Current Endocrine Therapy		
Tamoxifen	5	25%
Aromatase inhibitor	10	50%
None	5	25%

Table 6. Clinical baseline characteristics of the sample (n=20), reported as absolute and relative frequencies

	Pre	Post	$p(F)$ (η_p^2)
	Median ± SD	**Median ± SD**	
BMI (kg/m^2)	23.99 ± 2.74	23.72 ± 2.32	0.311 (0.054)
Fat mass (%)	29.15 ± 4.78	29.11 ± 3.76	0.952 (0.000)
$\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	32.55 ± 4.54	35.78 ± 6.07	<0.001 (0.617)
Fasting glucose (mg/dL)	100.15 ± 10.84	90.45 ± 11.55	<0.001 (0.597)
Testosterone (ng/mL)	0.28 ± 0.14	0.21 ± 0.17	0.018 (0.259)
Insulin (microU/mL)	6.99 ± 4.27	5.69 ± 3.73	0.091 (0.143)
HOMA index	1.81 ± 1.44	1.32 ± 1.02	0.036 (0.211)
Progesterone (ng/mL)	0.47 ± 0.25	0.47 ± 0.22	0.977 (0.000)
Triglycerides (mg/dL)	102.60 ± 43.68	87.02 ± 43.29	0.018 (0.263)
Total cholesterol (mg/dL)	207.10 ± 31.99	197.85 ± 33.31	0.119 (0.123)
HDL (mg/dL)	59.15 ± 14.37	58.10 ± 11.89	0.539 (0.020)
LDL (mg/dL)	129.90 ± 23.44	120.75 ± 26.98	0.022 (0.246)
Hs-CRP (mg/L)	1.30 ± 0.84	1.07 ± 0.88	0.078 (0.155)
Mediterranean diet score	7.65 ± 2.32	9.25 ± 2.38	0.002 (0.406)

Table 7. Variations in cardiorespiratory fitness, body composition, and blood biomarkers.
BMI, body mass index; $\dot{V}O_{2max}$, maximal oxygen uptake; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; Mediterranean diet score: MeDiet Score by DianaWeb.

4.3.2. Microbial composition

After the lifestyle intervention, as shown in **Figure 19**, there was a significant (p=0.025) increase in OTUs number. The pre-intervention count was 212.0 (Q1: 155.8; Q3 = 242.0), while the post-intervention number was 224.5 (Q1: 151.5; Q3: 252.2), which represents a 5.7% increase. However, there was no significant (p=0.620) change in the ENS as measured by Shannon's ENS index. The ENS

count remained relatively constant, with a pre-intervention count of 37.2 (Q1: 25.4; Q3: 45.7) and a post-intervention count of 36.8 (Q1: 21.5; Q3: 44.2).

PERMANOVA revealed significant differences between pre and post-intervention at phylum (F=0.62; p=0.035) and order levels (F=0.51; p=0.036). *Post-hoc* comparisons at the phylum level detected a significant reduction in the abundance of Proteobacteria (pre: 3.01% (1.45-4.43), post: 2.22% (1.03-2.80); V=189; p=0.006, ES=0.701), while no significant changes were reported for any of the other phyla (**Figure 20**).

Although not significant (p=0.067), a moderate effect-size (ES=0.410) increase in the Firmicutes/Bacteroidetes (F/B) ratio could be noticed, which showed values of 1.19 (0.82-1.43) in the pre-intervention and 1.24 (0.99-1.55) in the post-intervention.

At the order level, significant changes were observed for Lactobacillales (p=0.040, ES=0.459), Acidaminococcales (p=0.048, ES=0.442), and Burkholderiales (p=0.005, ES=0.609). However, these p-values become not significant when applying FDR correction for multiple comparisons (p=0.380 for Lactobacillales and Acidaminococcales, p=0.110 for Burkholderiales).

At the genus level, significant decreases in *Odoribacter* (p=0.024), *Erysipelotrichaceae_UCG-003* (p=0.020), *Coprococcus* (p=0.042), *Lachnospiraceae_UCG-004* (p=0.015), *Sutterella* (p=0.007) and a significant increase in *Colidextribacter* (p=0.044) were observed. However, when an exploratory non-metric multidimensional scaling was used to compare global genera abundances at the two times, ellipses were almost perfectly overlapping (**Figure 21**), supporting the absence of significant results in the PERMANOVA.

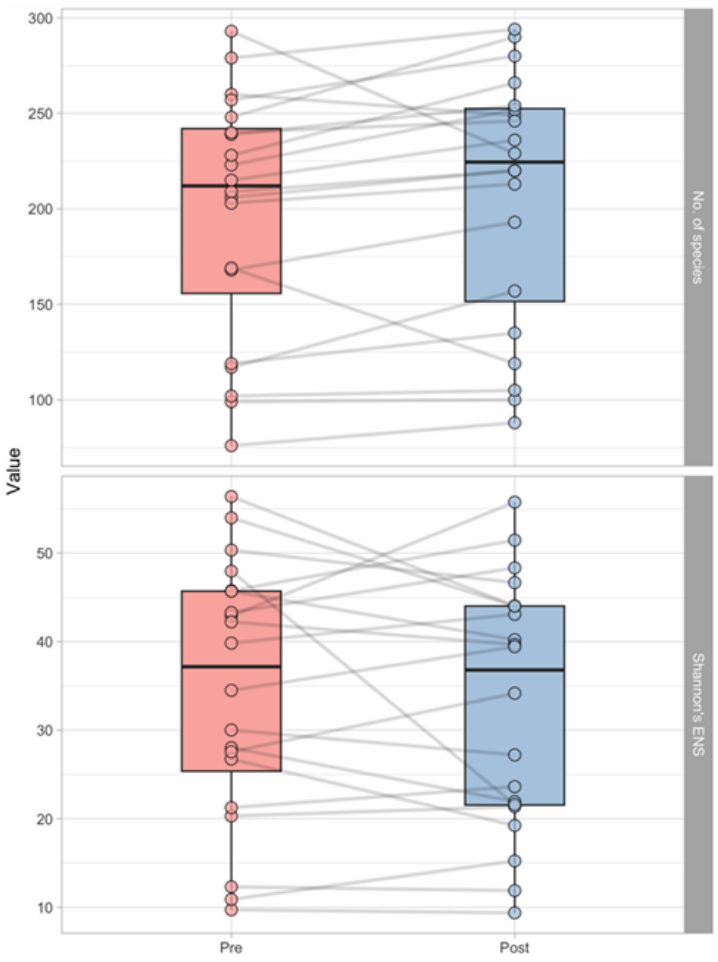


Figure 19. Boxplots representing the number of operational taxonomic units (OUT's number, above) and Shannon's effective number of species (ENS, below) in pre- and post-intervention, respectively

Each point represent a subject, and grey lines connect pre and post-intervention measurements of each participant.

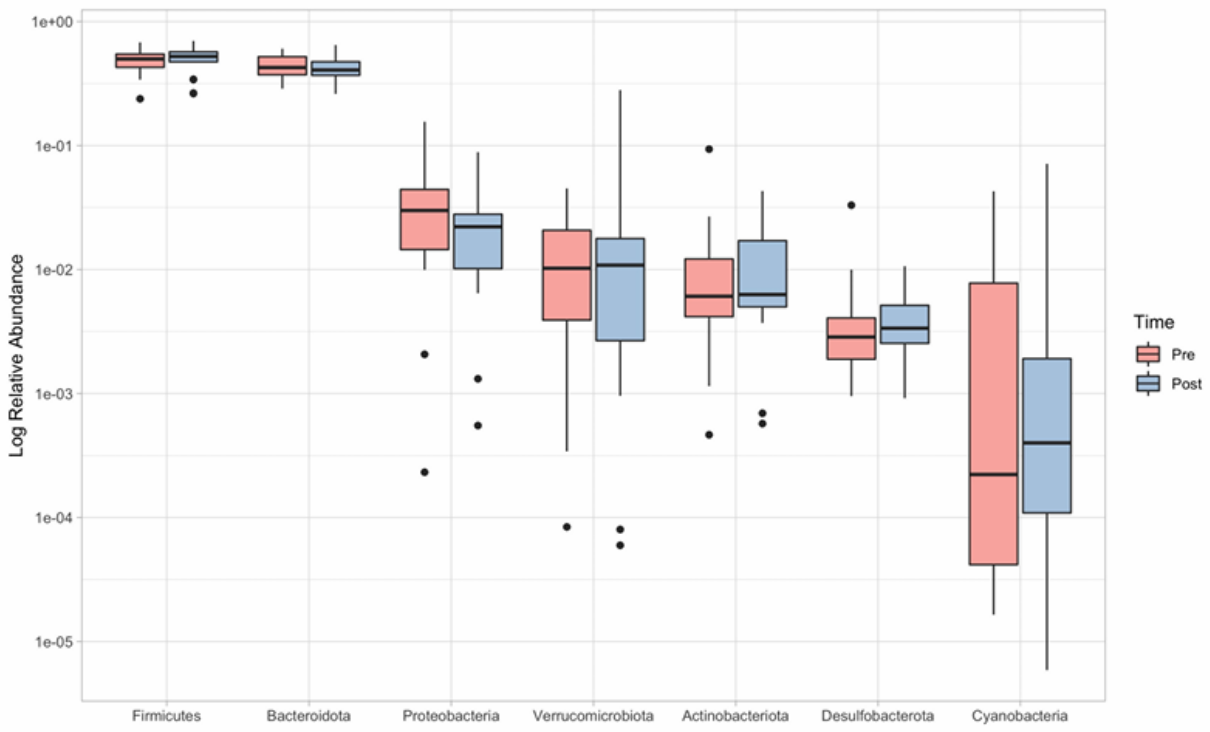


Figure 20. Relative abundances (log scale) of the seven more representative phyla in order of abundance

Boxes limits represent 25th and 75th percentiles, and the blank line inside the box represents the median. Single dots represent outliers individual data, which fall outside the 95% confidence interval.

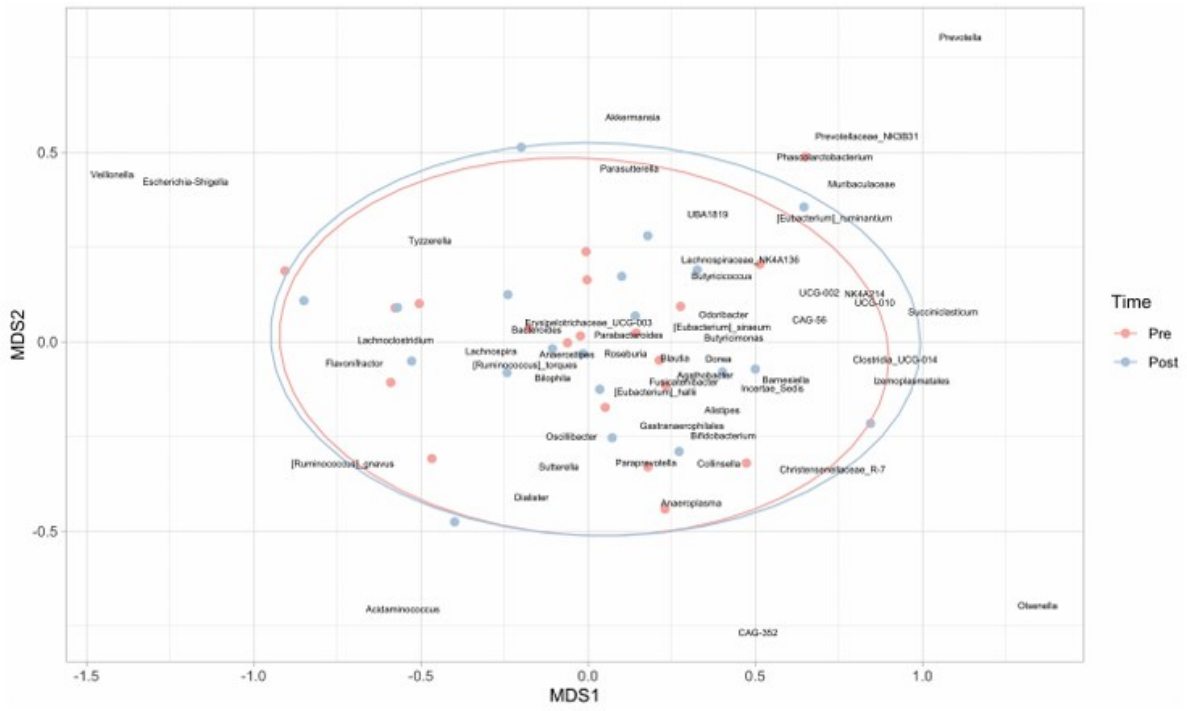


Figure 21. **Non-metric multidimensional scaling (nMDS) at the genera level**

Red points represent subjects in the pre-intervention, while blue dots refer to the post-intervention; 95% confidence ellipses are also represented.

4.3.3. Correlations with physical variables

A correlation matrix has been used to explore associations between variations of anthropometric and cardiometabolic variables and genera abundances (**Figure 22**). Negative correlations were reported between fasting glucose and *Clostridia_vadinBB60* (r=-0.62), insulin and HOMA index and *Butyricicoccus* genera (r = -0.72 and -0.66, respectively), and HDL cholesterol and *Escherichia/Shigella* (r = -0.59). Moreover, positive correlations were found between MD adherence and *Lachnospiraceae_ND3007* (r = 0.50), *Faecalibacterium* (r = 0.38), and *Butyricimonas* (r = 0.39).

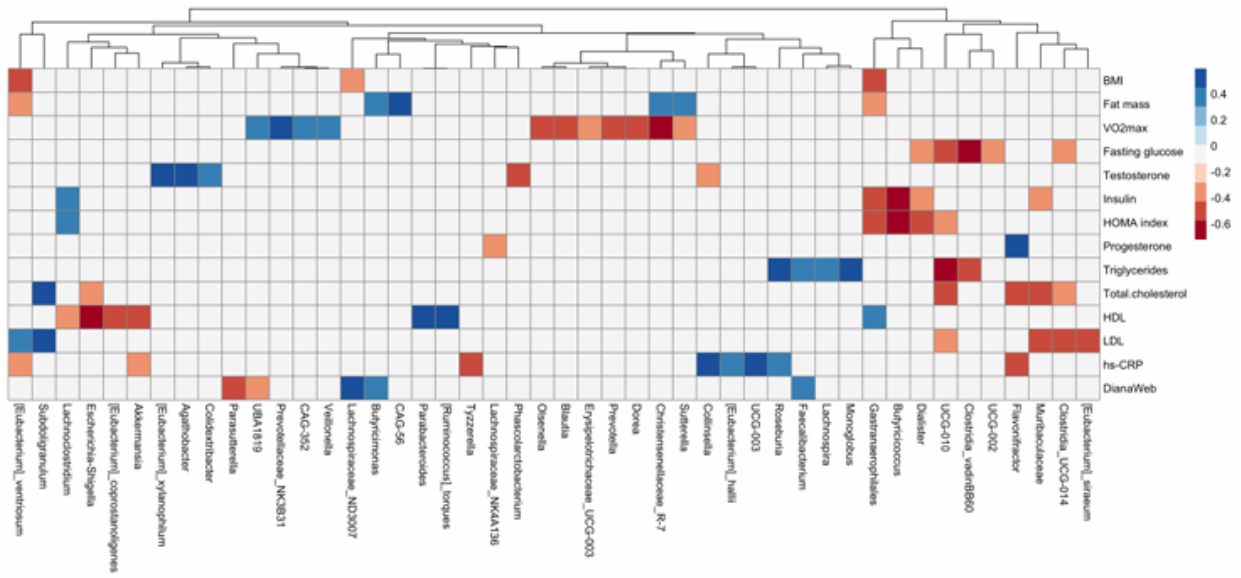


Figure 22. Correlation plot between delta (post-pre) values of anthropometric and cardiometabolic variables (body composition, cardiorespiratory fitness, and blood biomarkers) and genera relative abundances

Column clustering among genera is reported. Blue color indicates positive correlation, red color negative correlation. Only variables showing significant correlations are reported (Spearman $r_{0.05.20} \geq 0.378$).

4.4 Discussion

The risk of cancer recurrence and the increased risk of chronic diseases brought on by cancer treatments endanger the well-being of cancer survivors [174–177]. Improving lifestyle behaviors attenuates these risks, and in particular, nutrition and physical activity have been considered powerful allies to cancer patients and survivors, as they positively impact body mass, physical fitness, fatigue, depressive symptoms, anxiety, inflammatory profile, and quality of life [174–178]. Inversely, unhealthy eating patterns and a sedentary lifestyle are the main contributors to metabolic syndrome, which is associated with chronic low-grade systemic inflammation and symptoms in patients with BC who have undergone chemotherapy and survivors [179–181].

Diet and exercise are known to alter the composition of the GM, with resulting health-improving effects [182,183]. Up to date, a limited number of human studies have uncovered that exercise could play a beneficial role in gut health, increasing microbial diversity and changing microbiota composition depending on training intensity and duration [184]. Only a few clinical studies

investigated the effects of dietary interventions on the gut microbiome and/or metabolome in patients with cancer [185].

In this study, we investigated the impact of a home-based lifestyle intervention on the GM of BC survivors, demonstrating remarkable changes. In particular, a significant decrease in Proteobacteria has been observed. This phylum is normally associated with the instability of the microbial community that can lead to dysbiosis or disease [72]. Furthermore, *Escherichia/Shigella* bacteria belonging to the Proteobacteria phylum possess β-glucuronidase enzymes, which produce free estrogens from conjugated estrogens derived from the liver; free estrogens can re-enter the bloodstream and reach different organs, including the breast [186].

Physical exercise has been previously reported to reduce inflammatory processes, decreasing Proteobacteria but also increasing Actinobacteria [184]. Though representing only a small percentage of the total microbial community, Actinobacteria play a key role in maintaining the homeostasis of the GIT by increasing the expression of occluding junctions, regulating the synthesis and catabolism of mucins, providing energy for epithelial cell proliferation, and stimulating the immune system. In particular, *Bifidobacteria* (belonging to the phylum Actinobacteria) produce lactate, which can be used by a group of microorganisms defined as "lactate users" to produce butyrate, an SCFA that plays an important role in regulating energy metabolism and insulin sensitivity [187]. Moreover, the F/B ratio increased after the lifestyle intervention, although not statistically significant. This result agrees with other studies in which increases in the F/B ratio occur following physical activity [152,184] and is particularly important since Firmicutes are the main producers of SCFA butyrate. Furthermore, a relationship between cardiorespiratory fitness ($\dot{V}O_{2max}$) and F/B ratio in healthy young adults was also reported by Durk et al. [188]. A slight but not significant increase was observed in Actinobacteria and Bifidobacteria after 12 weeks of a home-based lifestyle intervention in BC survivors, which exerts an inflammatory and beneficial metabolic effect [189]. Interestingly, at the order level, Lactobacillales were found to be significantly increased after the lifestyle intervention. This order includes bacteria belonging to the genus of *Lactobacillus*, which are microorganisms with anti-inflammatory functions and may have a role against BC, as already suggested [190]. Moreover, the lifestyle intervention produced a decrease in *Sutterella*, a bacterial genus previously found enriched in patients with BC [191]. The correlation analysis between bacterial genera and anthropometric and cardiometabolic variables evidenced a strong positive association between adherence to MD and the butyrate

producers *Lachnospiraceae_ND3007*, *Faecalibacterium*, and *Butyricimonas*, in agreement with previous studies [192,193].

An increase in the consumption of dried fruit and cereal-based meals, and a reduction of red meat, desserts, and glasses of wine per week (data not shown) have been observed in all participants. The improvement of the MeDiet score obtained from the lifestyle intervention is particularly interesting for glycemic control, as highlighted by the amelioration of metabolic and inflammatory parameters (**Table 7**). The MD has been linked to improved health, including the production of SCFAs and anti-inflammatory properties that reduce the risk of chronic inflammatory diseases such as type II diabetes [194–196].

Another butyrate-producing microorganism, *Butyricicoccus*, was negatively correlated with insulin levels and the HOMA index. This result is in line with previous studies demonstrating that butyrate acts as an insulin-sensitizing agent [197,198]. Moreover, *Butyricicoccus* was previously found to be enriched in physically active people with respect to less active individuals, as reported by Magzdal et al. [199]. The same was observed for *Clostridia_vadinBB60* which, in our study, was negatively correlated with fasting glucose. An interesting inverse association was found between the pro-inflammatory bacteria *Escherichia/Shigella* and HDL cholesterol.

The present study shows that a lifestyle intervention based on physical activity and adherence to the MD in patients with BC was able to shape GM towards a healthier profile by modulating some microorganisms capable of decreasing inflammation and others capable of improving the lipid and glycemic assets of the host. The study recognizes certain limitations. Due to the relatively small sample size, the data are preliminary and should be interpreted with caution. Also, the absence of a control group should be acknowledged. Even though significant changes have been noted, it is still unknown whether also tiny modifications have any biological significance. Nonetheless, it's among the earliest studies which demonstrate how a lifestyle intervention in previously sedentary BC survivors can lead to an improvement in the composition of their gut microbial community. In order to gain a more comprehensive understanding of this topic, further research is required.

This study is part of the MoviS Trial (NCT04818359), a randomized controlled trial on the effect of aerobic exercise training on quality of life among BC survivors starting in January 2020 and ending in 2025. The forced changes in the study protocol, imposed by COVID-19 pandemic restrictions, made the difference between IA and CA interventions for the first recruited group of BC survivors negligible, and the results of the two groups were combined [168] qualifying this study as a pre-post assessment. Although the lack of a control group represents a limitation, no healthy control group,

and no No-Covid control group were included, further studies on the MoviS cohort will allow a comparison of these preliminary data obtained on GM and verify whether some other factors (e.g., COVID-19 restrictions) could better explain the changes in the GM induced by a 12-weeks lifestyle intervention in this particular BC survivors population.

In this study, we enrolled BC survivors at high risk of recurrence due to their sedentary condition, metabolic syndrome, inflammatory level, and dysmetabolism in the pandemic emergency, and we verified the effect of a lifestyle intervention in the improvement of cardio-metabolic condition and microbiota in a pre- and post-intervention assessment.

The elaborations presented in this study should be contextualized, taking into account the type of BC survivor population in Italy during the first Covid lockdown that tended to aggravate the BC health status by worsening their lifestyle and, consequently, their prognosis. In this line, we verified, for example, the profile of sedentary behavior among 781 BC survivors belonging to the cohort of the Italian DianaWeb project in the period of the first Covid lockdown. We observed a trend toward a decrease in physical activity (22%, 57%, and 26% for walking activity, vigorous activity, and total PA, respectively), and the sitting and lying time increased up to 54.2% [167], suggesting how COVID-19 emergency increased the unhealthy behaviors in BC patients, indicative of a possible higher risk of worse prognosis.

Our findings suggest that adopting a healthy lifestyle, by affecting the GM, may help to improve some biological parameters, such as the reduction of the inflammatory pattern involved in health maintenance, improvement of the prognosis, and reduction of the recurrence risk.

5. Microbiota-Derived Propionate as a Modulator of EMT and Tumor Progression in NSCLC

5.1 Background

Non-small cell lung cancer (NSCLC) remains one of the most lethal malignancies worldwide, with an estimated 5-year survival rate of only 20–30%. Despite advances in targeted therapies and immunotherapy, prognosis remains poor, highlighting the urgent need for novel biological approaches to modulate tumor aggressiveness and therapeutic response. Emerging evidence has revealed a critical role of microbiota in cancer biology. Microbial dysbiosis has been increasingly associated with enhanced tumor growth, invasiveness, and immune evasion [200]. In the context of lung cancer, preclinical studies have demonstrated that lung-resident bacteria can directly influence tumor progression and aggressiveness, promoting oncogenic signaling and modulating the tumor immune microenvironment [201].

Lung tissue was traditionally regarded as a sterile environment, devoid of microbial colonization under healthy conditions [202]. However, recent metagenomic and culture-based evidence has overturned this paradigm, showing that continuous microaspiration and aerosolized bacterial dispersal from the upper respiratory tract maintain a distinct lung microbiota. This microbial community engages in dynamic metabolic crosstalk with host tissues, contributing to physiological homeostasis in health (eubiosis) and shifting toward a pro-inflammatory, tumor-promoting state in disease (dysbiosis) [203]. Notably, specific bacterial taxa produce SCFAs—such as butyrate, acetate, and propionate—that exert profound effects on host cell metabolism and gene expression. Recent studies have shown that these microbial metabolites can reprogram tumor gene expression and suppress cancer aggressiveness in vivo [204]. These findings open new perspectives for the development of microbiota-driven therapeutic strategies, particularly those employing non-pathogenic, LPS-free bacterial strains capable of producing beneficial metabolites without triggering deleterious immune activation.

The main goal of this project is to investigate the potential of microbially mediated biotherapeutic approaches in the context of lung cancer. Specifically, the study aims to characterize and control the microbial production of key anticancer metabolites—such as SCFAs—with the ultimate objective of developing a translational framework for microbiota-based interventions against NSCLC. In this context, *Acidipropionibacterium microaerophilum* has been selected as the bacterial strain of

interest to evaluate its capacity to produce SCFAs and to assess their potential protective effects on A549 lung cancer cells, particularly in modulating tumor progression through the inhibition of epithelial-to-mesenchymal transition (EMT). This approach seeks to harness the metabolic versatility of commensal bacteria as a novel therapeutic tool to modulate tumor biology, enhance treatment responsiveness, and ultimately improve clinical outcomes in patients with lung cancer.

5.1.1. Non-small cell lung cancer (NSCLC)

Lung cancer is traditionally divided into two major histological subtypes: small-cell lung cancer (SCLC) and NSCLC. The latter accounts for approximately 85% of all lung cancer cases.

NSCLC encompasses three predominant histological subtypes distinguished by their microscopic features:

- Adenocarcinoma, originating from mucus-secreting glandular cells,
- Squamous cell carcinoma, derived from the bronchial squamous epithelium,
- Large cell carcinoma, a heterogeneous group of poorly differentiated epithelial tumors.

Less common histological variants include adenosquamous carcinomas, displaying both glandular and squamous features, and sarcomatoid carcinomas, characterized by the coexistence of epithelial and mesenchymal components

Tobacco smoking remains the leading global risk factor for lung cancer. Despite a decline in smoking prevalence over the past two decades, in 2020 more than 20% of the world's population were still smokers, with a prevalence of 15.4% among individuals aged 15–24 years. However, the incidence of lung cancer in never-smokers has been steadily increasing, emphasizing the need to explore additional etiological factors. Exposure to carcinogens, whether from tobacco or environmental sources, induces genomic alterations that promote uncontrolled cell growth and malignant transformation [205].

According to GLOBOCAN (2022), lung cancer was the most frequently diagnosed malignancy worldwide, with approximately 2.5 million new cases (12.4% of all cancers), and represented the leading cause of cancer-related death (18.7%). Incidence and mortality are nearly twice as high in men as in women, although the male-to-female ratio varies widely across regions. In the United States, both incidence and mortality have declined in recent decades for both sexes, whereas in Europe mortality has decreased among men but increased among women, likely reflecting differences in historical smoking patterns (**Figure 23**) [206].

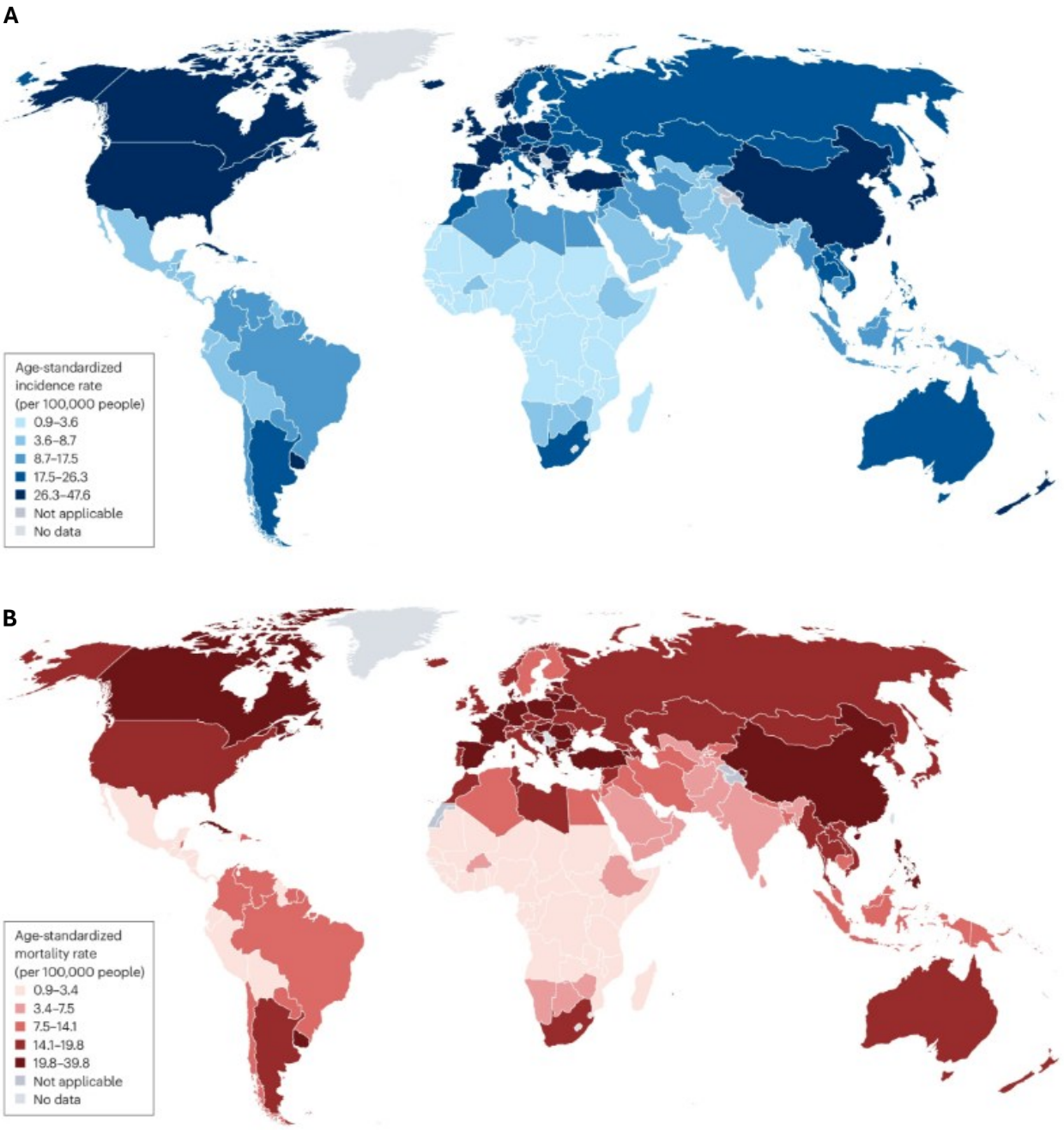


Figure 23. Global maps present (A) the incidence rate and (B) the mortality rate

The sizes of the respective populations are included in the legend [206].

Sex-related disparities are also evident in clinical presentation: women diagnosed with lung cancer tend to be younger, have fewer comorbidities, and are more frequently never-smokers compared with men. The median age at diagnosis is approximately 71 years, and nearly half of patients present with advanced-stage disease at the time of diagnosis. NSCLC constitutes the majority of lung cancer cases, with adenocarcinoma (≈50%) and squamous cell carcinoma (20–30%) being the most

prevalent subtypes, often associated with smoking history and specific druggable genomic alterations

Lung carcinogenesis may originate from two principal sites within the respiratory tract (**Figure 24**):

1. The peripheral bronchioloalveolar epithelium, where the terminal respiratory unit undergoes a multistep process from atypical adenomatous hyperplasia and adenocarcinoma in situ to invasive adenocarcinoma,
2. The central bronchial epithelium, where progressive changes from squamous dysplasia and carcinoma in situ lead to invasive squamous cell carcinoma.

Only a minority of precancerous lesions progress to invasive carcinoma. Rarely, adenocarcinomas may arise centrally, and squamous carcinomas may develop in the lung periphery. Moreover, adenocarcinomas can partially or completely transform into squamous carcinomas during tumor evolution.

In patients with interstitial lung diseases, abnormal proliferation of lung epithelium carries an increased risk of malignant transformation into either adenocarcinoma or squamous cell carcinoma. Among never-smokers, adenocarcinoma is the most frequent histological subtype and often harbors targetable genomic alterations, such as EGFR mutations or ALK, ROS1, and RET fusions. Conversely, tumors in smokers tend to be genetically more complex, most frequently involving KRAS and BRAF mutations. Squamous cell carcinomas also exhibit marked genomic complexity, though druggable oncogenic alterations remain uncommon [205].

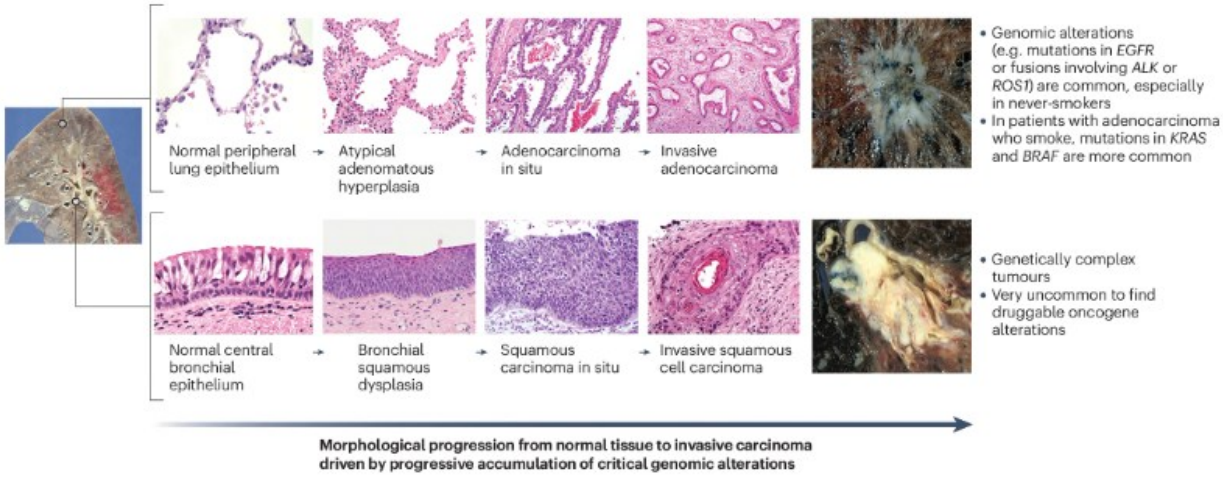


Figure 24. Carcinogenesis occurs in two main tissues in the lung [205]

Over the past two decades, thoracic oncology has undergone remarkable progress, particularly in the management of NSCLC. Advances span from early detection and screening to the introduction of novel systemic therapies, including immune checkpoint inhibitors (ICIs) and personalized targeted therapies for oncogene-addicted NSCLC. Notably, both therapeutic strategies are increasingly being applied to early-stage disease, leading to improved outcomes across all stages.

A deeper understanding of the mechanisms of acquired resistance has facilitated the development of next-generation agents capable of overcoming therapeutic resistance, thereby expanding treatment options for selected patients. Local treatment modalities have also evolved: refinements in surgical techniques have reduced postoperative morbidity, while advances in radiation therapy have improved precision and minimized toxicity. This has enabled the use of local ablative treatments, particularly in patients with oligometastatic NSCLC.

Despite these major achievements, global disparities in access to care persist, resulting in substantial geographical differences in disease burden and clinical outcomes [205].

5.1.2 Lung microbiome

The lung microbiome differs substantially from other microbial ecosystems of the human body, such as those inhabiting the gut or the oral cavity. It is characterized by a low microbial biomass and by a dynamic balance between microbial immigration and clearance, resulting in a fluid and variable composition rather than a stable community structure [202].

The upper respiratory tract — comprising the nasal cavity, paranasal sinuses, pharynx, and supraglottic portion of the larynx — is richly colonized by bacteria, with distinct topographical variations in microbial composition. In the nasal cavity and nasopharynx, dominant genera include *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Haemophilus*, and Streptococcus, whereas the oropharynx is mainly populated by *Prevotella*, *Veillonella*, *Leptotrichia*, *Rothia*, *Neisseria*, and *Haemophilus*.

In contrast, the lower respiratory tract — including the trachea and lungs — harbors a much lower microbial biomass. This condition is maintained by efficient physiological clearance mechanisms, which are crucial for preserving the primary respiratory function of oxygen and carbon dioxide exchange (**Figure 25**).

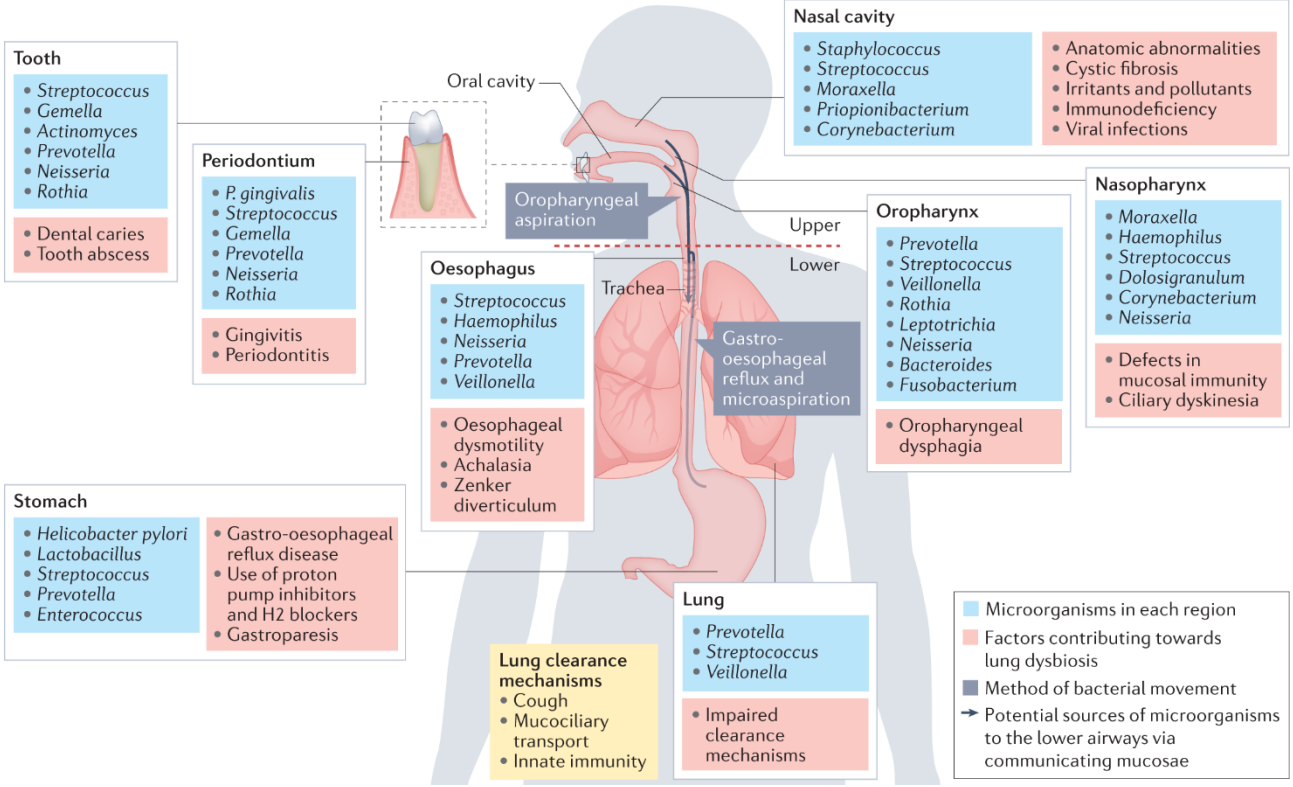


Figure 25. Overview of the microbial distribution across the respiratory tract [202]

Alterations in the normal microbial balance can exert detrimental biological effects and significantly influence the development and progression of respiratory diseases, underscoring the clinical importance of understanding the biology of the lung microbiome.

In recent years, the application of advanced culture-independent genomic techniques has enabled a more detailed characterization of the lung microbial environment. These studies have revealed that specific microbial signatures are associated with distinct inflammatory endotypes of the lower airways and with particular pathophysiological processes.

The lung microbiome should therefore be regarded as a dynamic system, constantly oscillating between states of low microbial presence and transient colonization. Experimental evidence, including mouse models of microaspiration, supports this dynamic nature, which clearly distinguishes the lung from high-biomass mucosal sites such as the oral cavity or the gut, where microbial communities display greater stability and resilience.

A deeper understanding of the dynamic behavior of the lung microbiome is essential to elucidate the pathogenetic mechanisms underlying pulmonary diseases and to identify novel therapeutic targets at the microbe–host interface [202].

5.1.3 Lung microbiome and lung cancer pathogenesis

The airway microbiota plays a crucial role in the initiation and progression of lung cancer. Experimental models have demonstrated that germ-free or antibiotic-treated mice are markedly protected against lung tumor development driven by Kirsten Rat Sarcoma Viral Oncogene Homolog (KRAS) mutation and Tumor Protein p53 (p53) loss [207]. Conversely, the presence of commensal microorganisms can stimulate inflammatory responses — particularly through the activation of γδ T cells — thereby promoting the formation of lung adenocarcinoma [201].

As proposed by McLean et al. [208], microbial dysbiosis may contribute to pulmonary carcinogenesis through three interrelated mechanisms:

1. Immune imbalance, where reduced microbial diversity limits antigen presentation and dampens antitumor immune activation, while excessive bacterial proliferation may lead to chronic immune overstimulation and expansion of interleukin-17 (IL-17)–producing $CD4^+$ T cells, key drivers of lung tumorigenesis.
2. Chronic inflammation, resulting from microbial metabolites and genotoxins that damage host DNA, as well as the release of reactive oxygen and nitrogen species by inflammatory cells, fostering angiogenesis and neoplastic transformation.
3. Direct activation of oncogenic signaling pathways by specific bacterial taxa. For example, exposure to microcystin, a cyanobacterial toxin, has been linked to PARP1 overexpression in NSCLC tissues, whereas *Streptococcus* and *Veillonella* species have been associated with activation of the Phosphoinositide 3-Kinase (PI3K) and Extracellular Signal-Regulated Kinase (ERK) pathways, both implicated in lung cancer pathogenesis.

Although increasing evidence connects certain microbial species to tumor-promoting mechanisms, it remains challenging to distinguish between microorganisms that actively drive oncogenesis and those that merely colonize the tumor microenvironment opportunistically. Clarifying this distinction is essential for advancing our understanding of the microbiota–cancer axis and for exploring the airway microbiome as a potential therapeutic target in lung cancer [201].

Building upon these immunological and inflammatory mechanisms, recent studies have broadened the understanding of how the lung microbiota influences tumor biology through additional metabolic, molecular, and microenvironmental pathways. The formation of a sustained inflammatory niche promotes neoplastic transformation, particularly in patients with chronic lung diseases such as COPD, where microbial dysbiosis perpetuates inflammation and structural parenchymal damage. Bacterial metabolites, N-formyl peptides, and cytokines such as interleukin-

6 (IL-6) and interleukin-8 (IL-8) activate the NF-κB signaling cascade, enhancing tumor cell proliferation, migration, and invasion.

Several microbial taxa have been linked to oncogenic signaling pathways:

- *Helicobacter pylori* promotes the release of IL-1, IL-6, and TNF-α, fostering a pro-tumorigenic inflammatory milieu.
- *Bacteroides fragilis* activates STAT3 via T-helper 17 cells, supporting tumor proliferation and angiogenesis, and secretes genotoxins such as cytolethal distending toxin that impair DNA repair.
- *Streptococcus* and *Veillonella* have been associated with activation of PI3K and ERK pathways, involved in both tumor initiation and metastatic progression.

Microbiota-induced metabolic reprogramming has also been reported. In patients with lung cancer, microbial alterations correlate with decreased energy metabolism and ATP-binding cassette transport, coupled with enhanced amino acid and lipid metabolism—changes that promote malignant transformation of airway epithelial cells. Among these molecular pathways, PI3K/AKT/mTOR plays a pivotal role, as its dysregulation drives uncontrolled proliferation, metabolic remodeling, and resistance to apoptosis.

Moreover, the microbiota modulates the immune microenvironment of the lung. In murine models of lung adenocarcinoma, dysbiosis stimulates γδ T-cell proliferation and IL-17 production, triggering local inflammation that supports tumor growth. Experimental reduction of microbial burden or inhibition of IL-17 signaling significantly limits tumor progression, underscoring the immunomodulatory impact of airway bacteria.

Beyond tumor initiation, the microbiota contributes to metastasis and angiogenesis. Pathogens such as *Mycobacterium tuberculosis* and *Haemophilus influenzae* activate pro-metastatic signaling (including TLR2 and PTPA) and promote neovascularization through mediators such as Vascular Endothelial Growth Factor (VEGF), IL-6, and IL-8. Other agents, including Helicobacter pylori, Human Papillomavirus type 16 (HPV-16), and Mycoplasma species, enhance tumor invasiveness and vascular remodeling through mechanisms involving PI3K/AKT and c-Jun signaling [209].

Collectively, these findings indicate that the airway microbiota influences lung carcinogenesis, tumor progression, and metastasis through the interplay of chronic inflammation, immune modulation, metabolic reprogramming, and oncogenic signaling activation. A deeper understanding of these interactions could pave the way for microbiota-targeted strategies in the prevention and therapy of lung cancer.

5.1.4 Metabolic Crosstalk between Tumor Cells and the Microbiome

The lung microbiota produces a wide range of bioactive metabolites, including SCFAs — particularly acetate, propionate, and butyrate — as well as lactate and methylglyoxal (MGO), derived from the fermentation of dietary substrates (**Figure 26**).

These molecules influence cellular metabolism and immune responses within the tumor microenvironment, modulating immune cell proliferation and antitumor activity.

Some of these metabolites, such as propionate, butyrate, and lactate, can also alter chromatin structure and regulate gene expression, exerting both pro- and anti-tumor effects depending on the cellular and metabolic context.

Overall, microbiota-derived metabolites create a complex network of metabolic interactions among tumor, microbial, and stromal cells, while also shaping immune cell function.

This interdependence can contribute either to the progression or inhibition of lung cancer and represents a promising therapeutic target to modulate both metabolism and immunity within the tumor microenvironment [209].

Among these metabolites, propionate has recently gained attention for its protective role in suppressing EMT and reducing metastatic potential in NSCLC [210].

EMT is a key biological process in which epithelial cells lose polarity and intercellular adhesion, acquiring mesenchymal features that enhance motility, invasiveness, and resistance to therapy.

Treatment with propionate has been shown to reinforce epithelial characteristics, sensitize tumor cells to cisplatin, and promote histone acetylation, suggesting that microbial metabolites may contribute to epigenetic remodeling and tumor suppression [204].

Based on this evidence, two propionate-producing bacterial species — *Lactobacillus brevis* and *Acidopropionibacterium microaerophilum* — were selected for the present study. Both taxa have been detected within the murine lung microbiota, indicating ecological relevance to the respiratory niche, and their metabolic activity provides a biologically meaningful model to investigate how microbially derived propionate may modulate EMT processes in NSCLC.

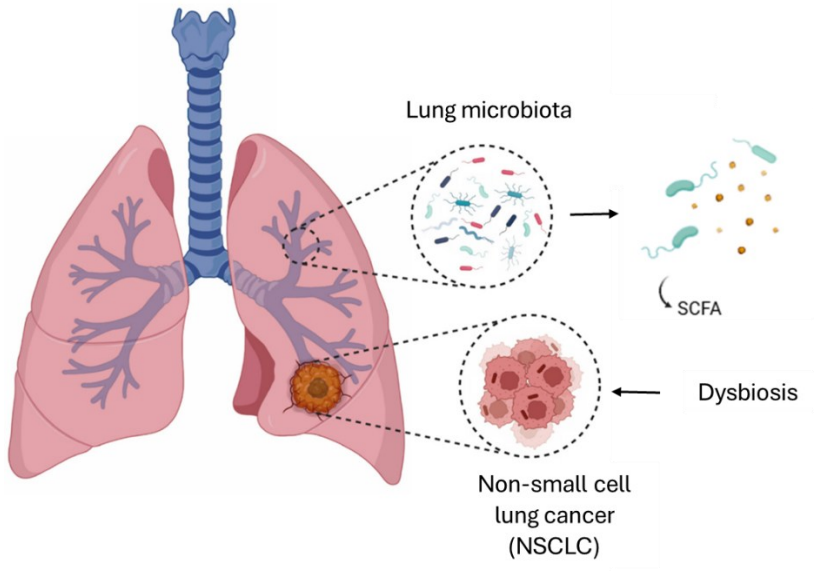


Figure 26. The crosstalk between the non-small cell lung cancer (NSCLC) and the lung microbiota

5.2 Methods

5.2.1 Bacterial cultures and growth conditions

Lactobacillus brevis (LB) and *Acidopropionibacterium microaerophilum* (AM DSMZ13435) are Gram-positive, facultatively anaerobic, and microaerophilic bacteria, meaning that they thrive in environments with low oxygen concentrations [211]. This metabolic versatility allows them to adapt to both aerobic and anaerobic conditions, making it particularly suitable for the controlled production of SCFAs under low oxygen levels.

Lactobacillus brevis (LB) was cultured in ZMB liquid medium containing 7.2 g/L glucose, 1 g/L yeast extract (w/v), 3.1 g/L KH_2PO_4, 6.4 g/L K_2HPO_4, 1 g/L $MgSO_4x7H_2O$, and supplemented with essential amino acids at various concentrations, adjusted to pH 6.5.

Strain AM DSMZ13435 was cultured in CYL liquid medium composed of 10 g/L casein peptone, 5 g/L yeast extract, and 14 mL/L sodium lactate (60% w/w; Sigma-Aldrich, L7900-100ML) as the main carbon source, adjusted to pH 7.0.

Both bacterial strains were incubated at 30 °C under two different conditions—static and shaking (70 rpm)—in their respective growth media (ZMB for LB and CYL for AM DSMZ13435), as well as in Dulbecco's Modified Eagle Medium (DMEM), to evaluate the most favorable environment for growth and metabolite production.

Optical density measurements (OD_{600}) were obtained and correlated with colony-forming units (CFU/mL) by determining cell counts through microscopic visualization using a hemocytometer.
Regular samples were collected from the cultures to monitor growth kinetics and to determine the exponential growth phase. This provided an estimate of the bacterial growth rate.
Additionally, supernatant samples were collected at different time intervals to quantify glucose, pyruvic acid, lactic acid, and SCFAs by HPLC equipped with a SUPELCOGEL C-610H column and a Refractive Index Detector (RID).
To mimic the physiological environment of lung epithelial cells, both LB and AM DSMZ13435 were also cultured in DMEM medium at 37 °C under static conditions. This setup was designed to assess bacterial adaptation, metabolic activity, and SCFA production in conditions comparable to those used for A549 cell culture assays.

5.2.2. A549 cell culture and treatment conditions

A549 cells, a human alveolar basal epithelial cell line derived from lung adenocarcinoma, were used for all experiments.
Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin (P/S), and 1% L-glutamine, and maintained at 37 °C in a humidified atmosphere containing 5% CO_2.
Prior to the bacterial treatment experiments, the culture medium was replaced with antibiotic-free DMEM to avoid interference with bacterial growth.
Before treatment, AM DSMZ13435 was re-inoculated in DMEM medium 4 days in advance, allowing bacterial adaptation to the mammalian culture environment and accumulation of secreted metabolic compounds in the medium.
A549 cells were seeded in 12-well plates and allowed to adhere for 24 hours. After removal of the growth medium, the wells were treated with filtered supernatants obtained from AM DSMZ13435 cultures grown in DMEM media, applied in serial twofold dilutions (1:2), and incubated for 18 hours. Following treatment, the medium was removed, and the cells were lysed by shaking for 5 minutes in RIPA buffer to obtain whole-cell lysates. Total protein concentration was then quantified using the bicinchoninic acid (BCA) assay.

The extracted protein lysates were subsequently processed for protein expression analysis, focusing on key epithelial and mesenchymal markers to evaluate the potential impact of AM DSMZ13435–derived metabolites on the EMT process.

5.2.3 Western Blot analysis

Protein lysates were prepared using Pierce RIPA lysis buffer (Thermo Scientific) supplemented with 1× Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific), following manufacturer's instructions. Total protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

Approximately 5 µg of total protein from each sample were resolved on a 10% SDS–PAGE gel and subsequently transferred onto a polyvinylidene difluoride (PVDF) membrane (Thermo Scientific). Membranes were blocked for 1 hour at room temperature in blocking buffer consisting of either 5% non-fat dry milk or 3% bovine serum albumin (BSA) prepared in 1× TBS-T (Tris-buffered saline with 0.1% Tween-20).

Following blocking, membranes were incubated overnight at 4 °C with the following primary antibodies:

- Zinc finger E-box-binding homeobox 1 (ZEB-1, rabbit polyclonal, diluted 1:2.000, BSA),
- Vimentin (VIM, rabbit polyclonal, diluted 1:3.000, milk),
- E-cadherin (CDH1, mouse monoclonal, diluted 1:5.000, milk),
- Tubulin Alpha 4A (TUBA4A, mouse monoclonal, diluted 1:10.000, milk) as the housekeeping protein.

The next day, membranes were washed three times with 1× TBS-T and incubated for 1 hour at room temperature with HRP-conjugated secondary antibodies (Southern Biotech) at a dilution of 1:10.000:

- Goat anti-mouse IgG-HRP (Southern Biotech, 1071-05)
- Goat anti-rabbit IgG-HRP (Southern Biotech, 4030-05).

Protein bands were visualized using Pierce ECL Western Blotting Substrate (Thermo Scientific) and detected with X-ray films (Thermo Scientific). Band intensity was quantified using ImageJ software, normalizing target protein levels to TUBA4A, and expressed as the relative fold change compared with control samples (**Figure 27**).

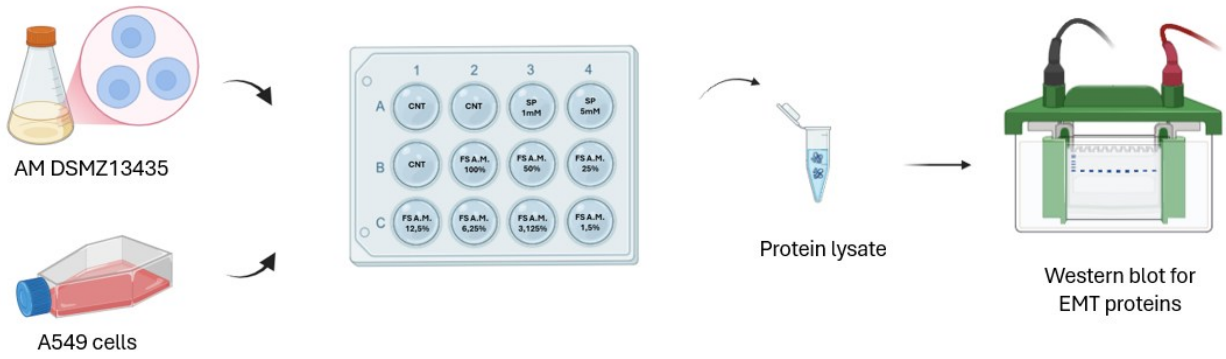


Figure 27. Workflow of the experimental design

5.3 Results

5.3.1. Bacterial growth dynamics and SCFA production

The growth profile of LB showed that the strain was able to proliferate efficiently under both ZMB and DMEM culture conditions (**Figure 28A**). In ZMB broth, LB produced detectable levels of propionic acid (PA) (1.2 g/L) after 24 hours of incubation at 70 rpm, confirming its metabolic capacity to synthesize SCFAs under low-oxygen and mild agitation conditions. Static conditions supported bacterial growth only during the initial 48 hours, after which shaking enhanced both cell proliferation and metabolite accumulation (**Figure 29A**). Furthermore, LB was capable of growing at 37 °C in DMEM medium (**Figure 28A**), demonstrating its adaptability to a mammalian-like environment and its potential to maintain metabolic activity under conditions similar to those of A549 cell culture.

AM DSMZ13435 exhibited a more robust and sustained growth profile in both CYL and DMEM media. OD_{600} values increased steadily over time, confirming its ability to grow efficiently not only in its standard medium but also in DMEM, which mimics the mammalian culture environment (**Figure 28B**). In CYL broth, AM DSMZ13435 produced substantial quantities of SCFAs, particularly acetic acid (AA) and PA, reaching concentrations of approximately 2.64 g/L and 5.5 g/L, respectively, after 192 hours of incubation at 70 rpm under microaerophilic conditions. Similarly, when cultured in DMEM, the strain maintained a comparable metabolic profile, producing increasing amounts of AA and PA up to 3 g/L between 48 and 168 hours (**Figure 29B**). Importantly,

AM DSMZ13435 was also able to grow at 37 °C in DMEM (**Figure 28B**), confirming its metabolic activity in conditions analogous to mammalian cell culture.

Given its higher metabolic output—particularly the elevated production of propionic acid under shaking conditions—AM DSMZ13435 was selected for subsequent experiments involving A549 cell treatment to evaluate the potential protective effects of its secreted metabolites on tumor progression and EMT.

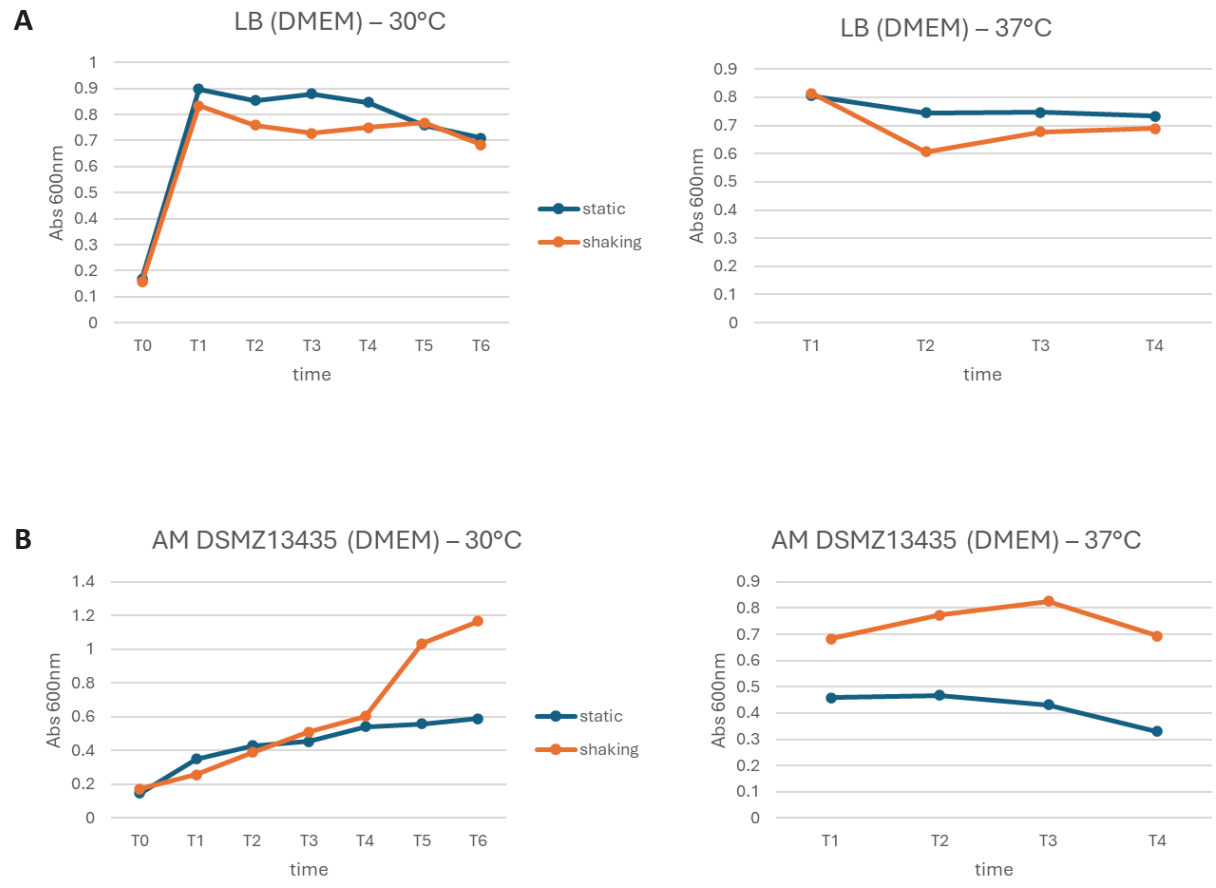


Figure 28. Growth curves of A) *Lactobacillus brevis* (LB) and **B)** *Acidopropionibacterium microaerophilum* (AM DSMZ13435) in DMEM at 30°C and 37°C, respectively.

Figure 29. HPLC results of *Lactobacillus brevis* (LB) and *Acidopropionibacterium microaerophilum* (AM DSMZ13435) under different conditions: A) ZMB e CYL media, respectivetely; **B)** DMEM medium.

5.3.2. Protein expression analysis — Western Blot results

Western blot analysis was performed to evaluate the modulation of EMT markers in A549 cells treated with filtered bacterial supernatants from AM DSMZ13435.

Protein lysates were obtained after 18 h of exposure, and expression levels of ZEB-1, E-cadherin, and VIM were quantified relative to the housekeeping protein TUBA4A.

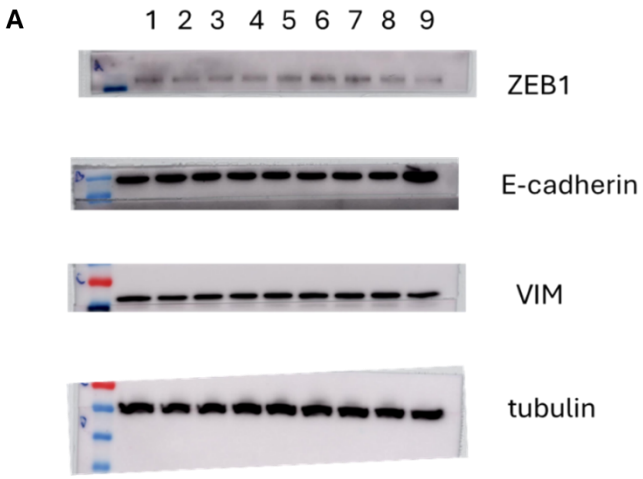
As shown in **Figure 30**, ZEB-1 expression exhibited a general trend toward downregulation across the tested dilutions, suggesting a partial suppression of EMT-related transcriptional activity. This downregulation was most pronounced in samples treated with the highest concentrations of bacterial supernatant, indicating a dose-dependent response.

Similarly, VIM, a key structural component of mesenchymal cells, demonstrated reduced expression in treated samples compared with controls, particularly at higher supernatant concentrations. This reduction is consistent with a reversion toward an epithelial phenotype characterized by decreased motility and invasive potential.

In contrast, E-cadherin, a hallmark epithelial adhesion protein and canonical marker of EMT inhibition, was found to be upregulated following treatment, with a more evident increase at higher doses of bacterial supernatant. The upregulation of E-cadherin, coupled with the suppression of ZEB-1 and Vimentin, supports the notion that AM DSMZ13435-derived metabolites exert a protective and anti-EMT effect in lung epithelial tumor cells.

Collectively, these findings indicate that exposure to AM DSMZ13435supernatants promotes a shift toward an epithelial phenotype by modulating key EMT markers in a direction opposite to that observed during mesenchymal transition.

This protective effect is particularly evident at higher supernatant concentrations, where the metabolic activity of the bacterium—most likely linked to the production of short-chain fatty acids, especially propionate—appears to play a pivotal role in maintaining epithelial integrity and counteracting EMT-driven tumor progression in A549 cells.



1. CNT
2. F.s. A.M. 100%
3. F.s. A.M. 50%
4. F.s. A.M. 25%
5. F.s. A.M. 12,5%
6. F.s. A.M. 6,25%
7. F.s. A.M. 3,1%
8. F.s. A.M. 1,5%
9. SP 5 mM

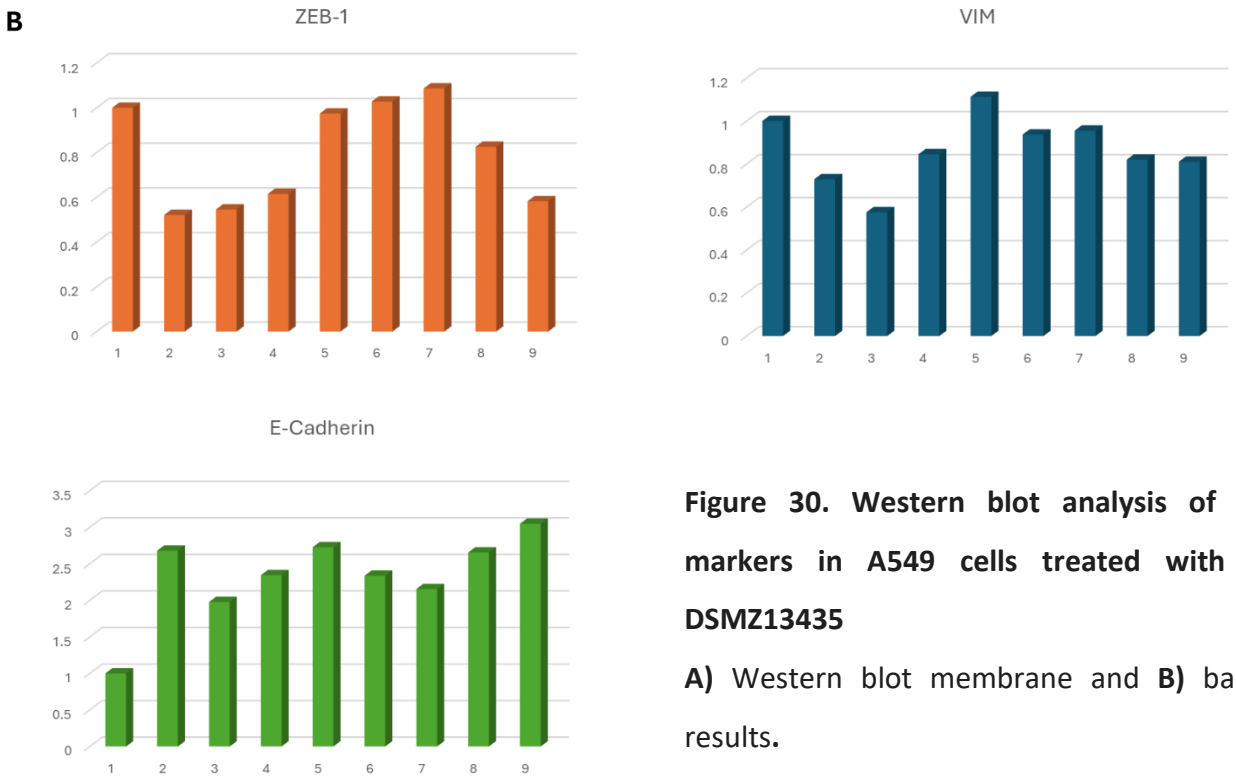


Figure 30. Western blot analysis of EMT markers in A549 cells treated with AM DSMZ13435

A) Western blot membrane and **B)** barplot results**.**

5.4 Discussion

This study investigated the potential of AM DSMZ13435 to produce bioactive SCFA and to modulate EMT in NSCLC through metabolic–epigenetic interactions. The bacterium demonstrated adaptability to both bacterial and mammalian-like conditions and released metabolites capable of influencing tumor cell behavior. In particular, the results indicate that exposure of A549 cells to bacterial

supernatants enriched in SCFA—especially propionate—was associated with a shift toward an epithelial phenotype, supporting an anti-EMT and potentially anti-metastatic effect.

The observed modulation of EMT markers is consistent with current evidence linking cellular metabolism to cancer cell plasticity. Metabolic reprogramming is now recognized as a major hallmark of EMT, and targeting these processes has gained increasing therapeutic attention [212,213]. In this context, SCFA such as propionate have emerged as regulators of epithelial identity, acting through mechanisms that include histone acetylation and chromatin remodeling [210,214]. Functional genomic studies in NSCLC identified a negative association between EMT activity and the presence of propionate and butyrate, supporting the idea that these metabolites may restrain mesenchymal differentiation and invasive potential.

The effect of propionate on EMT appears to involve epigenetic reprogramming via p300-mediated histone H3 lysine 27 acetylation (H3K27ac) enrichment, leading to the activation of epithelial genes such as CDH1 and the suppression of transcriptional repressors including ZEB1 [204,215,216]. These findings are consistent with the molecular trends observed in this study, suggesting that AM DSMZ13435–derived SCFA can contribute to the reinforcement of epithelial features in lung cancer cells. In addition, propionate has been shown to sensitize tumor cells to chemotherapy and reduce metastatic dissemination, further underscoring its potential therapeutic relevance [217,218].

The biological implications of these findings extend beyond EMT regulation. SCFA have been implicated in the modulation of immune and inflammatory pathways [219], maintenance of epithelial barrier function [220], and regulation of host–microbiota metabolic crosstalk [221]. The ability of AM DSMZ13435 to produce SCFA under mammalian-compatible conditions supports the concept that lung-associated or exogenous microbial species could influence the tumor microenvironment through metabolic and epigenetic signaling.

Overall, this study provides preliminary evidence that AM DSMZ13435–derived metabolites, particularly propionate, can counteract EMT-associated phenotypes in NSCLC cells, suggesting a link between microbial metabolism and tumor suppression. These findings contribute to the growing recognition of the microbiota as a modifiable factor in cancer biology, offering potential for translational applications in metabolic or probiotic-based adjuvant therapies.

Further work should aim to elucidate the molecular mechanisms by which propionate influences chromatin dynamics and EMT transcription factors, and to assess the in vivo relevance of these effects in NSCLC models. Ultimately, leveraging microbial metabolic pathways for therapeutic

benefit could provide a novel avenue for the prevention or treatment of EMT-driven cancer progression and fibrosis.

6. Conclusion

The relationship between GM and host physiology is increasingly recognized as dynamic and bidirectional, influencing metabolic balance, immune function, and neuroendocrine regulation. Changes in microbial communities are consistently observed in conditions related to chronic inflammation, neurodegeneration, metabolic dysfunction, and tumor progression. However, it remains unclear whether these shifts act as drivers, modulators, or consequences of disease.

What is becoming evident is that microbial metabolites, barrier integrity, and immune signaling are critical points of interaction through which the microbiota can impact systemic outcomes.

Interventions aimed at modifying the microbiota—whether through diet, physical activity, or the targeted use of microbial metabolites—present promising avenues for maintaining or restoring physiological balance. Nonetheless, individual variability, context-dependent responses, and the complexity of host-microbe co-regulation underscore the necessity for approaches that are personalized and deeply grounded in mechanisms. The ability of specific microbial products to influence cellular plasticity and inflammatory responses further suggests potential therapeutic applications, especially in situations where metabolic reprogramming and immune dysregulation are central concerns.

Despite these insights, several questions remain unanswered. The timing and sequence linking microbial shifts to the onset and progression of disease are not fully understood. The mechanisms by which microbial metabolites influence epigenetic and signaling pathways require further exploration. Additionally, translating microbial modulation into stable, clinically effective strategies will necessitate rigorous, long-term investigation.

Nonetheless, the intersection of microbiology, metabolism, immunology, and systems biology continues to redefine our understanding of health and disease. As profiling and manipulation tools for microbial ecosystems become increasingly precise, the microbiota offers both a lens for viewing complex pathophysiology and a potential target for interventions aimed at enhancing resilience, reducing chronic inflammation, and modulating disease trajectories.

7. References

1. Kelsen, J.R.; Wu, G.D. The Gut Microbiota, Environment and Diseases of Modern Society. *Gut Microbes* 2012, *3*, 374–382, doi:10.4161/gmic.21333.

2. Noecker, C.; Turnbaugh, P.J. Emerging Tools and Best Practices for Studying Gut Microbial Community Metabolism. *Nat Metab* 2024, *6*, 1225–1236, doi:10.1038/s42255-024-01074-z.

3. Clemente, J.C.; Ursell, L.K.; Parfrey, L.W.; Knight, R. The Impact of the Gut Microbiota on Human Health: An Integrative View. *Cell* 2012, *148*, 1258–1270, doi:10.1016/j.cell.2012.01.035.

4. Lloyd-Price, J.; Abu-Ali, G.; Huttenhower, C. The Healthy Human Microbiome. *Genome Med* 2016, *8*, 51, doi:10.1186/s13073-016-0307-y.

5. Bartolacci, A.; Stocchi, F.; Stocchi, V.; Zeppa, S.D. The Crucial Role of Vitamin D in Regulating Gut Microbiota in Inflammatory Bowel Disease. *Recent Progress in Nutrition* 2025, *05*, 1–8, doi:10.21926/rpn.2502007.

6. Petersen, C.; Round, J.L. Defining Dysbiosis and Its Influence on Host Immunity and Disease. *Cell Microbiol* 2014, *16*, 1024–1033, doi:10.1111/cmi.12308.

7. Ge, Y.; Wang, X.; Guo, Y.; Yan, J.; Abuduwaili, A.; Aximujiang, K.; Yan, J.; Wu, M. Gut Microbiota Influence Tumor Development and Alter Interactions with the Human Immune System. *Journal of Experimental & Clinical Cancer Research* 2021, *40*, 42, doi:10.1186/s13046-021-01845-6.

8. Chen, Y.; Zhou, J.; Wang, L. Role and Mechanism of Gut Microbiota in Human Disease. *Front Cell Infect Microbiol* 2021, *11*, doi:10.3389/fcimb.2021.625913.

9. Pedroza Matute, S.; Iyavoo, S. Exploring the Gut Microbiota: Lifestyle Choices, Disease Associations, and Personal Genomics. *Front Nutr* 2023, *10*, doi:10.3389/fnut.2023.1225120.

10. Virgin, H.W.; Todd, J.A. Metagenomics and Personalized Medicine. *Cell* 2011, *147*, 44–56, doi:10.1016/j.cell.2011.09.009.

11. Davenport, E.R.; Sanders, J.G.; Song, S.J.; Amato, K.R.; Clark, A.G.; Knight, R. The Human Microbiome in Evolution. *BMC Biol* 2017, *15*, 127, doi:10.1186/s12915-017-0454-7.

12. Turnbaugh, P.J.; Ley, R.E.; Hamady, M.; Fraser-Liggett, C.M.; Knight, R.; Gordon, J.I. The Human Microbiome Project. *Nature* 2007, *449*, 804–810, doi:10.1038/nature06244.

13. Peterson, J.; Garges, S.; Giovanni, M.; McInnes, P.; Wang, L.; Schloss, J.A.; Bonazzi, V.; McEwen, J.E.; Wetterstrand, K.A.; Deal, C.; et al. The NIH Human Microbiome Project. *Genome Res* 2009, *19*, 2317–2323, doi:10.1101/gr.096651.109.

14. Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K.S.; Manichanh, C.; Nielsen, T.; Pons, N.; Levenez, F.; Yamada, T.; et al. A Human Gut Microbial Gene Catalogue Established by Metagenomic Sequencing. *Nature* 2010, *464*, 59–65, doi:10.1038/nature08821.

15. Laterza, L.; Rizzatti, G.; Gaetani, E.; Chiusolo, P.; Gasbarrini, A. THE GUT MICROBIOTA AND IMMUNE SYSTEM RELATIONSHIP IN HUMAN GRAFT-VERSUS-HOST DISEASE. *Mediterr J Hematol Infect Dis* 2016, *8*, 2016025, doi:10.4084/mjhid.2016.025.

16. Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggiano, G.A.D.; Gasbarrini, A.; Mele, M.C. What Is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms* 2019, *7*, 14, doi:10.3390/microorganisms7010014.

17. Spellerberg, I.F.; Fedor, P.J. A Tribute to Claude Shannon (1916–2001) and a Plea for More Rigorous Use of Species Richness, Species Diversity and the 'Shannon–Wiener' Index. *Global Ecology and Biogeography* 2003, *12*, 177–179, doi:10.1046/j.1466-822X.2003.00015.x.

18. Bidell, M.R.; Hobbs, A.L. V.; Lodise, T.P. Gut Microbiome Health and Dysbiosis: A Clinical Primer. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* 2022, *42*, 849–857, doi:10.1002/phar.2731.

19. Arumugam, M.; Raes, J.; Pelletier, E.; Le Paslier, D.; Yamada, T.; Mende, D.R.; Fernandes, G.R.; Tap, J.; Bruls, T.; Batto, J.-M.; et al. Enterotypes of the Human Gut Microbiome. *Nature* 2011, *473*, 174–180, doi:10.1038/nature09944.

20. Wu, G.D.; Chen, J.; Hoffmann, C.; Bittinger, K.; Chen, Y.-Y.; Keilbaugh, S.A.; Bewtra, M.; Knights, D.; Walters, W.A.; Knight, R.; et al. Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. *Science (1979)* 2011, *334*, 105–108, doi:10.1126/science.1208344.

21. Ghannoum, M.A.; Jurevic, R.J.; Mukherjee, P.K.; Cui, F.; Sikaroodi, M.; Naqvi, A.; Gillevet, P.M. Characterization of the Oral Fungal Microbiome (Mycobiome) in Healthy Individuals. *PLoS Pathog* 2010, *6*, e1000713, doi:10.1371/journal.ppat.1000713.

22. Scanlan, P.D.; Marchesi, J.R. Micro-Eukaryotic Diversity of the Human Distal Gut Microbiota: Qualitative Assessment Using Culture-Dependent and -Independent Analysis of Faeces. *ISME J* 2008, *2*, 1183–1193, doi:10.1038/ismej.2008.76.

23. Breitbart, M.; Haynes, M.; Kelley, S.; Angly, F.; Edwards, R.A.; Felts, B.; Mahaffy, J.M.; Mueller, J.; Nulton, J.; Rayhawk, S.; et al. Viral Diversity and Dynamics in an Infant Gut. *Res Microbiol* 2008, *159*, 367–373, doi:10.1016/j.resmic.2008.04.006.

24. Haynes, M.; Rohwer, F. The Human Virome. In *Metagenomics of the Human Body*; Springer New York: New York, NY, 2011; pp. 63–77.

25. Minot, S.; Sinha, R.; Chen, J.; Li, H.; Keilbaugh, S.A.; Wu, G.D.; Lewis, J.D.; Bushman, F.D. The Human Gut Virome: Inter-Individual Variation and Dynamic Response to Diet. *Genome Res* 2011, *21*, 1616–1625, doi:10.1101/gr.122705.111.

26. Reyes, A.; Haynes, M.; Hanson, N.; Angly, F.E.; Heath, A.C.; Rohwer, F.; Gordon, J.I. Viruses in the Faecal Microbiota of Monozygotic Twins and Their Mothers. *Nature* 2010, *466*, 334–338, doi:10.1038/nature09199.

27. Https://En.Wikipedia.Org/Wiki/Human_digestive_system.

28. Mifkovic, A.; El Hassoun, O.; Lukacikova, P.; Malakhov, S.; Polák, S. The Intestine – Anatomy, Immunology, Barrier Function, Microbiome and Central Regulations. *European Journal of Anatomy* 2025, *29*, 287–302, doi:10.52083/SUQE1748.

29. Mifkovic, A.; El Hassoun, O.; Lukacikova, P.; Malakhov, S.; Polák, S. The Intestine – Anatomy, Immunology, Barrier Function, Microbiome and Central Regulations. *European Journal of Anatomy* 2025, *29*, 287–302, doi:10.52083/SUQE1748.

30. Neurath, M.F.; Artis, D.; Becker, C. The Intestinal Barrier: A Pivotal Role in Health, Inflammation, and Cancer. *Lancet Gastroenterol Hepatol* 2025, *10*, 573–592, doi:10.1016/S2468-1253(24)00390-X.

31. Di Tommaso, N.; Gasbarrini, A.; Ponziani, F.R. Intestinal Barrier in Human Health and Disease. *Int J Environ Res Public Health* 2021, *18*, 12836, doi:10.3390/ijerph182312836.

32. Ley, R.E.; Peterson, D.A.; Gordon, J.I. Ecological and Evolutionary Forces Shaping Microbial Diversity in the Human Intestine. *Cell* 2006, *124*, 837–848, doi:10.1016/j.cell.2006.02.017.

33. Rita Canella *Scienza Della Nutrizione. Fisiologia. Principali Patologie. Nutraceutica. Nutrigenomica*; Edi. Ermes, Ed.; Edi. Ermes: Milano, 2020;

34. Makino, H.; Kushiro, A.; Ishikawa, E.; Muylaert, D.; Kubota, H.; Sakai, T.; Oishi, K.; Martin, R.; Ben Amor, K.; Oozeer, R.; et al. Transmission of Intestinal Bifidobacterium Longum Subsp. *Longum* Strains from Mother to Infant, Determined by Multilocus Sequencing Typing and Amplified Fragment Length Polymorphism. *Appl Environ Microbiol* 2011, *77*, 6788–6793, doi:10.1128/AEM.05346-11.

35. Makino, H.; Kushiro, A.; Ishikawa, E.; Kubota, H.; Gawad, A.; Sakai, T.; Oishi, K.; Martin, R.; Ben-Amor, K.; Knol, J.; et al. Mother-to-Infant Transmission of Intestinal Bifidobacterial Strains Has an Impact on the Early Development of Vaginally Delivered Infant's Microbiota. *PLoS One* 2013, *8*, e78331, doi:10.1371/journal.pone.0078331.

36. Reyman, M.; van Houten, M.A.; van Baarle, D.; Bosch, A.A.T.M.; Man, W.H.; Chu, M.L.J.N.; Arp, K.; Watson, R.L.; Sanders, E.A.M.; Fuentes, S.; et al. Author Correction: Impact of Delivery Mode-Associated Gut Microbiota Dynamics on Health in the First Year of Life. *Nat Commun* 2019, *10*, 5352, doi:10.1038/s41467-019-13373-1.

37. Shao, Y.; Forster, S.C.; Tsaliki, E.; Vervier, K.; Strang, A.; Simpson, N.; Kumar, N.; Stares, M.D.; Rodger, A.; Brocklehurst, P.; et al. Stunted Microbiota and Opportunistic Pathogen Colonization in Caesarean-Section Birth. *Nature* 2019, *574*, 117–121, doi:10.1038/s41586-019-1560-1.

38. Yang, R.; Gao, R.; Cui, S.; Zhong, H.; Zhang, X.; Chen, Y.; Wang, J.; Qin, H. Dynamic Signatures of Gut Microbiota and Influences of Delivery and Feeding Modes during the First 6 Months of Life. *Physiol Genomics* 2019, *51*, 368–378, doi:10.1152/physiolgenomics.00026.2019.

39. Kuang, Y.-S.; Li, S.-H.; Guo, Y.; Lu, J.-H.; He, J.-R.; Luo, B.-J.; Jiang, F.-J.; Shen, H.; Papasian, C.J.; Pang, H.; et al. Composition of Gut Microbiota in Infants in China and Global Comparison. *Sci Rep* 2016, *6*, 36666, doi:10.1038/srep36666.

40. Wampach, L.; Heintz-Buschart, A.; Fritz, J. V.; Ramiro-Garcia, J.; Habier, J.; Herold, M.; Narayanasamy, S.; Kaysen, A.; Hogan, A.H.; Bindl, L.; et al. Birth Mode Is Associated with Earliest Strain-Conferred Gut Microbiome Functions and Immunostimulatory Potential. *Nat Commun* 2018, *9*, 5091, doi:10.1038/s41467-018-07631-x.

41. Nagpal, R.; Yamashiro, Y. Gut Microbiota Composition in Healthy Japanese Infants and Young Adults Born by C-Section. *Ann Nutr Metab* 2018, *73*, 4–11, doi:10.1159/000490841.

42. Akagawa, S.; Tsuji, S.; Onuma, C.; Akagawa, Y.; Yamaguchi, T.; Yamagishi, M.; Yamanouchi, S.; Kimata, T.; Sekiya, S.; Ohashi, A.; et al. Effect of Delivery Mode and Nutrition on Gut Microbiota in Neonates. *Ann Nutr Metab* 2019, *74*, 132–139, doi:10.1159/000496427.

43. Adlerberth, I.; Wold, A. Establishment of the Gut Microbiota in Western Infants. *Acta Paediatr* 2009, *98*, 229–238, doi:10.1111/j.1651-2227.2008.01060.x.

44. Bäckhed, F.; Roswall, J.; Peng, Y.; Feng, Q.; Jia, H.; Kovatcheva-Datchary, P.; Li, Y.; Xia, Y.; Xie, H.; Zhong, H.; et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe* 2015, *17*, 690–703, doi:10.1016/j.chom.2015.04.004.

45. Hill, C.J.; Lynch, D.B.; Murphy, K.; Ulaszewska, M.; Jeffery, I.B.; O'Shea, C.A.; Watkins, C.; Dempsey, E.; Mattivi, F.; Tuohy, K.; et al. Evolution of Gut Microbiota Composition from Birth to 24 Weeks in the INFANTMET Cohort. *Microbiome* 2017, *5*, 4, doi:10.1186/s40168-016-0213-y.

46. Dominguez-Bello, M.G.; Costello, E.K.; Contreras, M.; Magris, M.; Hidalgo, G.; Fierer, N.; Knight, R. Delivery Mode Shapes the Acquisition and Structure of the Initial Microbiota across Multiple Body Habitats in Newborns. *Proceedings of the National Academy of Sciences* 2010, *107*, 11971–11975, doi:10.1073/pnas.1002601107.

47. Martin, A.M.; Sun, E.W.; Rogers, G.B.; Keating, D.J. The Influence of the Gut Microbiome on Host Metabolism Through the Regulation of Gut Hormone Release. *Front Physiol* 2019, *10*, doi:10.3389/fphys.2019.00428.

48. Azad, M.; Konya, T.; Persaud, R.; Guttman, D.; Chari, R.; Field, C.; Sears, M.; Mandhane, P.; Turvey, S.; Subbarao, P.; et al. Impact of Maternal Intrapartum Antibiotics, Method of Birth and Breastfeeding on Gut Microbiota during the First Year of Life: A Prospective Cohort Study. *BJOG* 2016, *123*, 983–993, doi:10.1111/1471-0528.13601.

49. Toscano, M.; De Grandi, R.; Grossi, E.; Drago, L. Role of the Human Breast Milk-Associated Microbiota on the Newborns' Immune System: A Mini Review. *Front Microbiol* 2017, *8*, doi:10.3389/fmicb.2017.02100.

50. Senn, V.; Bassler, D.; Choudhury, R.; Scholkmann, F.; Righini-Grunder, F.; Vuille-dit-Bille, R.N.; Restin, T. Microbial Colonization From the Fetus to Early Childhood—A Comprehensive Review. *Front Cell Infect Microbiol* 2020, *10*, doi:10.3389/fcimb.2020.573735.

51. Benno, Y.; Sawada, K.; Mitsuoka, T. The Intestinal Microflora of Infants: Composition of Fecal Flora in Breast-Fed and Bottle-Fed Infants. *Microbiol Immunol* 1984, *28*, 975–986, doi:10.1111/j.1348-0421.1984.tb00754.x.

52. Bezirtzoglou, E.; Tsiotsias, A.; Welling, G.W. Microbiota Profile in Feces of Breast- and Formula-Fed Newborns by Using Fluorescence in Situ Hybridization (FISH). *Anaerobe* 2011, *17*, 478–482, doi:10.1016/j.anaerobe.2011.03.009.

53. Fallani, M.; Amarri, S.; Uusijarvi, A.; Adam, R.; Khanna, S.; Aguilera, M.; Gil, A.; Vieites, J.M.; Norin, E.; Young, D.; et al. Determinants of the Human Infant Intestinal Microbiota after the Introduction of First Complementary Foods in Infant Samples from Five European Centres. *Microbiology (N Y)* 2011, *157*, 1385–1392, doi:10.1099/mic.0.042143-0.

54. Stewart, C.J.; Ajami, N.J.; O'Brien, J.L.; Hutchinson, D.S.; Smith, D.P.; Wong, M.C.; Ross, M.C.; Lloyd, R.E.; Doddapaneni, H.; Metcalf, G.A.; et al. Temporal Development of the Gut Microbiome in Early Childhood from the TEDDY Study. *Nature* 2018, *562*, 583–588, doi:10.1038/s41586-018-0617-x.

55. Levin, A.M.; Sitarik, A.R.; Havstad, S.L.; Fujimura, K.E.; Wegienka, G.; Cassidy-Bushrow, A.E.; Kim, H.; Zoratti, E.M.; Lukacs, N.W.; Boushey, H.A.; et al. Joint Effects of Pregnancy, Sociocultural, and Environmental Factors on Early Life Gut Microbiome Structure and Diversity. *Sci Rep* 2016, *6*, 31775, doi:10.1038/srep31775.

56. Martin, A.M.; Sun, E.W.; Rogers, G.B.; Keating, D.J. The Influence of the Gut Microbiome on Host Metabolism Through the Regulation of Gut Hormone Release. *Front Physiol* 2019, *10*, doi:10.3389/fphys.2019.00428.

57. Eckburg, P.B.; Bik, E.M.; Bernstein, C.N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S.R.; Nelson, K.E.; Relman, D.A. Diversity of the Human Intestinal Microbial Flora. *Science (1979)* 2005, *308*, 1635–1638, doi:10.1126/science.1110591.

58. Ley, R.E.; Turnbaugh, P.J.; Klein, S.; Gordon, J.I. Human Gut Microbes Associated with Obesity. *Nature* 2006, *444*, 1022–1023, doi:10.1038/4441022a.

59. Turnbaugh, P.J.; Hamady, M.; Yatsunenko, T.; Cantarel, B.L.; Duncan, A.; Ley, R.E.; Sogin, M.L.; Jones, W.J.; Roe, B.A.; Affourtit, J.P.; et al. A Core Gut Microbiome in Obese and Lean Twins. *Nature* 2009, *457*, 480–484, doi:10.1038/nature07540.

60. Palmer, C.; Bik, E.M.; DiGiulio, D.B.; Relman, D.A.; Brown, P.O. Development of the Human Infant Intestinal Microbiota. *PLoS Biol* 2007, *5*, e177, doi:10.1371/journal.pbio.0050177.

61. Del Chierico, F.; Vernocchi, P.; Petrucca, A.; Paci, P.; Fuentes, S.; Praticò, G.; Capuani, G.; Masotti, A.; Reddel, S.; Russo, A.; et al. Phylogenetic and Metabolic Tracking of Gut Microbiota during Perinatal Development. *PLoS One* 2015, *10*, e0137347, doi:10.1371/journal.pone.0137347.

62. Koenig, J.E.; Spor, A.; Scalfone, N.; Fricker, A.D.; Stombaugh, J.; Knight, R.; Angenent, L.T.; Ley, R.E. Succession of Microbial Consortia in the Developing Infant Gut Microbiome. *Proceedings of the National Academy of Sciences* 2011, *108*, 4578–4585, doi:10.1073/pnas.1000081107.

63. Penders, J.; Thijs, C.; Vink, C.; Stelma, F.F.; Snijders, B.; Kummeling, I.; van den Brandt, P.A.; Stobberingh, E.E. Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy. *Pediatrics* 2006, *118*, 511–521, doi:10.1542/peds.2005-2824.

64. Yatsunenko, T.; Rey, F.E.; Manary, M.J.; Trehan, I.; Dominguez-Bello, M.G.; Contreras, M.; Magris, M.; Hidalgo, G.; Baldassano, R.N.; Anokhin, A.P.; et al. Human Gut Microbiome Viewed across Age and Geography. *Nature* 2012, *486*, 222–227, doi:10.1038/nature11053.

65. Bäckhed, F.; Fraser, C.M.; Ringel, Y.; Sanders, M.E.; Sartor, R.B.; Sherman, P.M.; Versalovic, J.; Young, V.; Finlay, B.B. Defining a Healthy Human Gut Microbiome: Current Concepts, Future Directions, and Clinical Applications. *Cell Host Microbe* 2012, *12*, 611–622, doi:10.1016/j.chom.2012.10.012.

66. Structure, Function and Diversity of the Healthy Human Microbiome. *Nature* 2012, *486*, 207–214, doi:10.1038/nature11234.

67. Kho, Z.Y.; Lal, S.K. The Human Gut Microbiome – A Potential Controller of Wellness and Disease. *Front Microbiol* 2018, *9*, doi:10.3389/fmicb.2018.01835.

68. Lawley, T.D.; Walker, A.W. Intestinal Colonization Resistance. *Immunology* 2013, *138*, 1–11, doi:10.1111/j.1365-2567.2012.03616.x.

69. Thursby, E.; Juge, N. Introduction to the Human Gut Microbiota. *Biochemical Journal* 2017, *474*, 1823–1836, doi:10.1042/BCJ20160510.

70. Tiffany, C.R.; Bäumler, A.J. Dysbiosis: From Fiction to Function. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 2019, *317*, G602–G608, doi:10.1152/ajpgi.00230.2019.

71. Weiss, G.A.; Hennet, T. Mechanisms and Consequences of Intestinal Dysbiosis. *Cellular and Molecular Life Sciences* 2017, *74*, 2959–2977, doi:10.1007/s00018-017-2509-x.

72. Shin, N.-R.; Whon, T.W.; Bae, J.-W. Proteobacteria: Microbial Signature of Dysbiosis in Gut Microbiota. *Trends Biotechnol* 2015, *33*, 496–503, doi:10.1016/j.tibtech.2015.06.011.

73. Kho, Z.Y.; Lal, S.K. The Human Gut Microbiome – A Potential Controller of Wellness and Disease. *Front Microbiol* 2018, *9*, doi:10.3389/fmicb.2018.01835.

74. Sanz, Y.; Cryan, J.F.; Deschasaux-Tanguy, M.; Elinav, E.; Lambrecht, R.; Veiga, P. The Gut Microbiome Connects Nutrition and Human Health. *Nat Rev Gastroenterol Hepatol* 2025, *22*, 534–555, doi:10.1038/s41575-025-01077-5.

75. Wastyk, H.C.; Fragiadakis, G.K.; Perelman, D.; Dahan, D.; Merrill, B.D.; Yu, F.B.; Topf, M.; Gonzalez, C.G.; Van Treuren, W.; Han, S.; et al. Gut-Microbiota-Targeted Diets Modulate Human Immune Status. *Cell* 2021, *184*, 4137-4153.e14, doi:10.1016/j.cell.2021.06.019.

76. Lotankar, M.; Houttu, N.; Mokkala, K.; Laitinen, K. Diet–Gut Microbiota Relations: Critical Appraisal of Evidence From Studies Using Metagenomics. *Nutr Rev* 2025, *83*, e1917–e1938, doi:10.1093/nutrit/nuae192.

77. Varghese, S.; Rao, S.; Khattak, A.; Zamir, F.; Chaari, A. Physical Exercise and the Gut Microbiome: A Bidirectional Relationship Influencing Health and Performance. *Nutrients* 2024, *16*, 3663, doi:10.3390/nu16213663.

78. Khaledi, M.; Darvishi, M.; Sameni, F.; Shahrjerdi, S.; Karami, E.; Barahui, N.; Hemmati, J.; Hasheminasab, M.S.; Sanae, M.-J.; Akhavan-Sigari, R.; et al. Association between Exercise and Changes in Gut Microbiota Profile: A Review. *Sport Sci Health* 2024, *20*, 273–286, doi:10.1007/s11332-023-01132-1.

79. Hart, N.H.; Wallen, M.P.; Farley, M.J.; Haywood, D.; Boytar, A.N.; Secombe, K.; Joseph, R.; Chan, R.J.; Kenkhuis, M.-F.; Buffart, L.M.; et al. Exercise and the Gut Microbiome: Implications for Supportive Care in Cancer. *Supportive Care in Cancer* 2023, *31*, 724, doi:10.1007/s00520-023-08183-7.

80. Collins, S.M.; Surette, M.; Bercik, P. The Interplay between the Intestinal Microbiota and the Brain. *Nat Rev Microbiol* 2012, *10*, 735–742, doi:10.1038/nrmicro2876.

81. Menozzi, E.; Schapira, A.H.V.; Borghammer, P. The Gut-Brain Axis in Parkinson Disease: Emerging Concepts and Therapeutic Implications. *Mov Disord Clin Pract* 2025, *12*, 904–916, doi:10.1002/mdc3.70029.

82. Hayes, M.T. Parkinson's Disease and Parkinsonism. *Am J Med* 2019, *132*, 802–807, doi:10.1016/j.amjmed.2019.03.001.

83. Beitz, J.M. Parkinson s Disease a Review. *Frontiers in Bioscience* 2014, *S6*, S415, doi:10.2741/S415.

84. Wang, Q.; Bu, C.; Wang, H.; Zhang, B.; Chen, Q.; Shi, D.; Chi, L. Distinct Mechanisms Underlying the Therapeutic Effects of Low-Molecular-Weight Heparin and Chondroitin Sulfate on Parkinson's Disease. *Int J Biol Macromol* 2024, *262*, 129846, doi:10.1016/j.ijbiomac.2024.129846.

85. Miller, I. The Gut–Brain Axis: Historical Reflections. *Microb Ecol Health Dis* 2018, *29*, 1542921, doi:10.1080/16512235.2018.1542921.

86. Whytt Robert *Observations on the Nature, Causes, and Cure of Those Disorders Which Have Been Commonly Called Nervous Hypochondriac, or Hysteric: To Which Are Prefixed Some Remarks on the Sympathy of the Nerves*; Becket T., Ed.; Edinburgh, 1765;

87. Cryan, J.F.; O'Riordan, K.J.; Cowan, C.S.M.; Sandhu, K. V.; Bastiaanssen, T.F.S.; Boehme, M.; Codagnone, M.G.; Cussotto, S.; Fulling, C.; Golubeva, A. V.; et al. The Microbiota-Gut-Brain Axis. *Physiol Rev* 2019, *99*, 1877–2013, doi:10.1152/physrev.00018.2018.

88. Sudo, N.; Chida, Y.; Aiba, Y.; Sonoda, J.; Oyama, N.; Yu, X.; Kubo, C.; Koga, Y. Postnatal Microbial Colonization Programs the Hypothalamic–Pituitary–Adrenal System for Stress Response in Mice. *J Physiol* 2004, *558*, 263–275, doi:10.1113/jphysiol.2004.063388.

89. Neufeld, K.M.; Kang, N.; Bienenstock, J.; Foster, J.A. Reduced Anxiety-like Behavior and Central Neurochemical Change in Germ-Free Mice. *Neurogastroenterology & Motility* 2011, *23*, 255-e119, doi:10.1111/j.1365-2982.2010.01620.x.

90. Bercik, P.; Denou, E.; Collins, J.; Jackson, W.; Lu, J.; Jury, J.; Deng, Y.; Blennerhassett, P.; Macri, J.; McCoy, K.D.; et al. The Intestinal Microbiota Affect Central Levels of Brain-Derived

Neurotropic Factor and Behavior in Mice. *Gastroenterology* 2011, *141*, 599-609.e3, doi:10.1053/j.gastro.2011.04.052.

91. Heijtz, R.D.; Wang, S.; Anuar, F.; Qian, Y.; Björkholm, B.; Samuelsson, A.; Hibberd, M.L.; Forssberg, H.; Pettersson, S. Normal Gut Microbiota Modulates Brain Development and Behavior. *Proceedings of the National Academy of Sciences* 2011, *108*, 3047–3052, doi:10.1073/pnas.1010529108.

92. Kim, S.; Kwon, S.-H.; Kam, T.-I.; Panicker, N.; Karuppagounder, S.S.; Lee, S.; Lee, J.H.; Kim, W.R.; Kook, M.; Foss, C.A.; et al. Transneuronal Propagation of Pathologic α-Synuclein from the Gut to the Brain Models Parkinson's Disease. *Neuron* 2019, *103*, 627-641.e7, doi:10.1016/j.neuron.2019.05.035.

93. Wang, Q.; Luo, Y.; Ray Chaudhuri, K.; Reynolds, R.; Tan, E.-K.; Pettersson, S. The Role of Gut Dysbiosis in Parkinson's Disease: Mechanistic Insights and Therapeutic Options. *Brain* 2021, *144*, 2571–2593, doi:10.1093/brain/awab156.

94. Reeve, A.; Simcox, E.; Turnbull, D. Ageing and Parkinson's Disease: Why Is Advancing Age the Biggest Risk Factor? *Ageing Res Rev* 2014, *14*, 19–30, doi:10.1016/j.arr.2014.01.004.

95. Sulzer, D. Multiple Hit Hypotheses for Dopamine Neuron Loss in Parkinson's Disease. *Trends Neurosci* 2007, *30*, 244–250, doi:10.1016/j.tins.2007.03.009.

96. Yamada, M.; Iwatsubo, T.; Mizuno, Y.; Mochizuki, H. Overexpression of A-synuclein in Rat Substantia Nigra Results in Loss of Dopaminergic Neurons, Phosphorylation of A-synuclein and Activation of Caspase-9: Resemblance to Pathogenetic Changes in Parkinson's Disease. *J Neurochem* 2004, *91*, 451–461, doi:10.1111/j.1471-4159.2004.02728.x.

97. Dickson, D.W. Parkinson's Disease and Parkinsonism: Neuropathology. *Cold Spring Harb Perspect Med* 2012, *2*, a009258–a009258, doi:10.1101/cshperspect.a009258.

98. Lim, S.; Lang, A.E. The Nonmotor Symptoms of Parkinson's Disease—An Overview. *Movement Disorders* 2010, *25*, doi:10.1002/mds.22786.

99. Lindvall, O.; Kokaia, Z. Prospects of Stem Cell Therapy for Replacing Dopamine Neurons in Parkinson's Disease. *Trends Pharmacol Sci* 2009, *30*, 260–267, doi:10.1016/j.tips.2009.03.001.

100. Golpich, M.; Amini, E.; Mohamed, Z.; Azman Ali, R.; Mohamed Ibrahim, N.; Ahmadiani, A. Mitochondrial Dysfunction and Biogenesis in Neurodegenerative Diseases: Pathogenesis and Treatment. *CNS Neurosci Ther* 2017, *23*, 5–22, doi:10.1111/cns.12655.

101. Braak, H.; Tredici, K. Del; Rüb, U.; de Vos, R.A.I.; Jansen Steur, E.N.H.; Braak, E. Staging of Brain Pathology Related to Sporadic Parkinson's Disease. *Neurobiol Aging* 2003, *24*, 197–211, doi:10.1016/S0197-4580(02)00065-9.

102. Braak, H.; de Vos, R.A.I.; Bohl, J.; Del Tredici, K. Gastric α-Synuclein Immunoreactive Inclusions in Meissner's and Auerbach's Plexuses in Cases Staged for Parkinson's Disease-Related Brain Pathology. *Neurosci Lett* 2006, *396*, 67–72, doi:10.1016/j.neulet.2005.11.012.

103. Braak, H.; de Vos, R.A.I.; Bohl, J.; Del Tredici, K. Gastric α-Synuclein Immunoreactive Inclusions in Meissner's and Auerbach's Plexuses in Cases Staged for Parkinson's Disease-Related Brain Pathology. *Neurosci Lett* 2006, *396*, 67–72, doi:10.1016/j.neulet.2005.11.012.

104. Liu, J.; Xu, F.; Nie, Z.; Shao, L. Gut Microbiota Approach—A New Strategy to Treat Parkinson's Disease. *Front Cell Infect Microbiol* 2020, *10*, doi:10.3389/fcimb.2020.570658.

105. Takahashi, K.; Nishiwaki, H.; Ito, M.; Iwaoka, K.; Takahashi, K.; Suzuki, Y.; Taguchi, K.; Yamahara, K.; Tsuboi, Y.; Kashihara, K.; et al. Altered Gut Microbiota in Parkinson's Disease Patients with Motor Complications. *Parkinsonism Relat Disord* 2022, *95*, 11–17, doi:10.1016/j.parkreldis.2021.12.012.

106. Mahbub, N.U.; Islam, M.M.; Hong, S.-T.; Chung, H.-J. Dysbiosis of the Gut Microbiota and Its Effect on α-Synuclein and Prion Protein Misfolding: Consequences for Neurodegeneration. *Front Cell Infect Microbiol* 2024, *14*, doi:10.3389/fcimb.2024.1348279.

107. Hirayama, M.; Ohno, K. Parkinson's Disease and Gut Microbiota. *Ann Nutr Metab* 2021, *77*, 28–35, doi:10.1159/000518147.

108. Disease Mechanisms as Subtypes: Microbiome. In; 2023; pp. 107–131.

109. Reunanen, S.; Ghemtio, L.; Patel, J.Z.; Patel, D.R.; Airavaara, K.; Yli-Kauhaluoma, J.; Jeltsch, M.; Xhaard, H.; Piepponen, P.T.; Tammela, P. Targeting Bacterial and Human Levodopa Decarboxylases for Improved Drug Treatment of Parkinson's Disease: Discovery and Characterization of New Inhibitors. *European Journal of Pharmaceutical Sciences* 2025, *211*, 107133, doi:10.1016/j.ejps.2025.107133.

110. Maini Rekdal, V.; Bess, E.N.; Bisanz, J.E.; Turnbaugh, P.J.; Balskus, E.P. Discovery and Inhibition of an Interspecies Gut Bacterial Pathway for Levodopa Metabolism. *Science (1979)* 2019, *364*, doi:10.1126/science.aau6323.

111. Clarke, C.E.; Patel, S.; Ives, N.; Rick, C.E.; Woolley, R.; Wheatley, K.; Walker, M.F.; Zhu, S.; Kandiyali, R.; Yao, G.; et al. Clinical Effectiveness and Cost-Effectiveness of Physiotherapy and Occupational Therapy versus No Therapy in Mild to Moderate Parkinson's Disease: A Large Pragmatic Randomised Controlled Trial (PD REHAB). *Health Technol Assess (Rockv)* 2016, *20*, 1–96, doi:10.3310/hta20630.

112. Julien, C.; Hache, G.; Dulac, M.; Dubrou, C.; Castelnovo, G.; Giordana, C.; Azulay, J.; Fluchère, F. The Clinical Meaning of Levodopa Equivalent Daily Dose in Parkinson's Disease. *Fundam Clin Pharmacol* 2021, *35*, 620–630, doi:10.1111/fcp.12646.

113. Modestino, E.J. Hoehn and Yahr Staging of Parkinson Rsquo s Disease in Relation to Neuropsychological Measures. *Frontiers in Bioscience* 2018, *23*, 4649, doi:10.2741/4649.

114. Opara, J.; Małecki, A.; Małecka, E.; Socha, T. Motor Assessment in Parkinson`s Disease. *Annals of Agricultural and Environmental Medicine* 2017, *24*, 411–415, doi:10.5604/12321966.1232774.

115. Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glöckner, F.O. Evaluation of General 16S Ribosomal RNA Gene PCR Primers for Classical and Next-

Generation Sequencing-Based Diversity Studies. *Nucleic Acids Res* 2013, *41*, e1–e1, doi:10.1093/nar/gks808.

116. Martin, M. Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads. *EMBnet J* 2011, *17*, 10, doi:10.14806/ej.17.1.200.

117. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-Resolution Sample Inference from Illumina Amplicon Data. *Nat Methods* 2016, *13*, 581–583, doi:10.1038/nmeth.3869.

118. Bokulich, N.A.; Kaehler, B.D.; Rideout, J.R.; Dillon, M.; Bolyen, E.; Knight, R.; Huttley, G.A.; Gregory Caporaso, J. Optimizing Taxonomic Classification of Marker-Gene Amplicon Sequences with QIIME 2's Q2-Feature-Classifier Plugin. *Microbiome* 2018, *6*, 90, doi:10.1186/s40168-018-0470-z.

119. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res* 2012, *41*, D590–D596, doi:10.1093/nar/gks1219.

120. Anderson, M.J. A New Method for Non-parametric Multivariate Analysis of Variance. *Austral Ecol* 2001, *26*, 32–46, doi:10.1111/j.1442-9993.2001.01070.pp.x.

121. Romano, S.; Savva, G.M.; Bedarf, J.R.; Charles, I.G.; Hildebrand, F.; Narbad, A. Meta-Analysis of the Parkinson's Disease Gut Microbiome Suggests Alterations Linked to Intestinal Inflammation. *NPJ Parkinsons Dis* 2021, *7*, 27, doi:10.1038/s41531-021-00156-z.

122. Zhang, X.; Tang, B.; Guo, J. Parkinson's Disease and Gut Microbiota: From Clinical to Mechanistic and Therapeutic Studies. *Transl Neurodegener* 2023, *12*, 59, doi:10.1186/s40035-023-00392-8.

123. Boertien, J.M.; Pereira, P.A.B.; Aho, V.T.E.; Scheperjans, F. Increasing Comparability and Utility of Gut Microbiome Studies in Parkinson's Disease: A Systematic Review. *J Parkinsons Dis* 2019, *9*, S297–S312, doi:10.3233/JPD-191711.

124. Elford, J.D.; Becht, N.; Garssen, J.; Kraneveld, A.D.; Perez-Pardo, P. Buty and the Beast: The Complex Role of Butyrate in Parkinson's Disease. *Front Pharmacol* 2024, *15*, doi:10.3389/fphar.2024.1388401.

125. Lubomski, M.; Xu, X.; Holmes, A.J.; Muller, S.; Yang, J.Y.H.; Davis, R.L.; Sue, C.M. The Gut Microbiome in Parkinson's Disease: A Longitudinal Study of the Impacts on Disease Progression and the Use of Device-Assisted Therapies. *Front Aging Neurosci* 2022, *14*, doi:10.3389/fnagi.2022.875261.

126. Nishiwaki, H.; Hamaguchi, T.; Ito, M.; Ishida, T.; Maeda, T.; Kashihara, K.; Tsuboi, Y.; Ueyama, J.; Shimamura, T.; Mori, H.; et al. Short-Chain Fatty Acid-Producing Gut Microbiota Is Decreased in Parkinson's Disease but Not in Rapid-Eye-Movement Sleep Behavior Disorder. *mSystems* 2020, *5*, doi:10.1128/mSystems.00797-20.

127. Hirayama, M.; Nishiwaki, H.; Hamaguchi, T.; Ohno, K. Gastrointestinal Disorders in Parkinson's Disease and Other Lewy Body Diseases. *NPJ Parkinsons Dis* 2023, *9*, 71, doi:10.1038/s41531-023-00511-2.

128. Koutsokostas, C.; Merkouris, E.; Goulas, A.; Aidinopoulou, K.; Sini, N.; Dimaras, T.; Tsiptsios, D.; Mueller, C.; Nystazaki, M.; Tsamakis, K. Gut Microbes Associated with Neurodegenerative Disorders: A Comprehensive Review of the Literature. *Microorganisms* 2024, *12*, 1735, doi:10.3390/microorganisms12081735.

129. Tao, Z.; Shi, A.; Lu, C.; Song, T.; Zhang, Z.; Zhao, J. Breast Cancer: Epidemiology and Etiology. *Cell Biochem Biophys* 2015, *72*, 333–338, doi:10.1007/s12013-014-0459-6.

130. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer Statistics, 2021. *CA Cancer J Clin* 2021, *71*, 7–33, doi:10.3322/caac.21654.

131. AIOM, A.O.N.S. and P. e P. d'argento, Siap.-I. *I Numeri Del Cancro in Italia 2022*; Brescia IT, Ed.; 2022;

132. Mangone, L.; Bisceglia, I.; Michiara, M.; Musolino, A.; Mazzoleni, G.; Caldarella, A.; Minerba, S.; Cascone, G.; Bella, F.; Dinaro, Y.; et al. Breast Cancer in Italy: Stage and Region Distribution. *Breast Cancer: Targets and Therapy* 2022, *Volume 14*, 125–131, doi:10.2147/BCTT.S360244.

133. Yang, J.; Tan, Q.; Fu, Q.; Zhou, Y.; Hu, Y.; Tang, S.; Zhou, Y.; Zhang, J.; Qiu, J.; Lv, Q. Gastrointestinal Microbiome and Breast Cancer: Correlations, Mechanisms and Potential Clinical Implications. *Breast Cancer* 2017, *24*, 220–228, doi:10.1007/s12282-016-0734-z.

134. Ruo, S.W.; Alkayyali, T.; Win, M.; Tara, A.; Joseph, C.; Kannan, A.; Srivastava, K.; Ochuba, O.; Sandhu, J.K.; Went, T.R.; et al. Role of Gut Microbiota Dysbiosis in Breast Cancer and Novel Approaches in Prevention, Diagnosis, and Treatment. *Cureus* 2021, doi:10.7759/cureus.17472.

135. Mikó, E.; Kovács, T.; Sebő, É.; Tóth, J.; Csonka, T.; Ujlaki, G.; Sipos, A.; Szabó, J.; Méhes, G.; Bai, P. Microbiome—Microbial Metabolome—Cancer Cell Interactions in Breast Cancer—Familiar, but Unexplored. *Cells* 2019, *8*, 293, doi:10.3390/cells8040293.

136. Sisti, D.; Pazienza, V.; Piccini, F.; Citterio, B.; Baffone, W.; Donati Zeppa, S.; Biavasco, F.; Prospero, E.; De Luca, A.; Artico, M.; et al. A Proposal for the Reference Intervals of the Italian Microbiota "Scaffold" in Healthy Adults. *Sci Rep* 2022, *12*, 3952, doi:10.1038/s41598-022-08000-x.

137. Donovan, S.M. Introduction to the Special Focus Issue on the Impact of Diet on Gut Microbiota Composition and Function and Future Opportunities for Nutritional Modulation of the Gut Microbiome to Improve Human Health. *Gut Microbes* 2017, *8*, 75–81, doi:10.1080/19490976.2017.1299309.

138. Zmora, N.; Suez, J.; Elinav, E. You Are What You Eat: Diet, Health and the Gut Microbiota. *Nat Rev Gastroenterol Hepatol* 2019, *16*, 35–56, doi:10.1038/s41575-018-0061-2.

139. Wang, F.; Roy, S. Gut Homeostasis, Microbial Dysbiosis, and Opioids. *Toxicol Pathol* 2017, *45*, 150–156, doi:10.1177/0192623316679898.

140. Anand, P.; Kunnumakara, A.B.; Sundaram, C.; Harikumar, K.B.; Tharakan, S.T.; Lai, O.S.; Sung, B.; Aggarwal, B.B. Cancer Is a Preventable Disease That Requires Major Lifestyle Changes. *Pharm Res* 2008, *25*, 2097–2116, doi:10.1007/s11095-008-9661-9.

141. Willett, W.; Sacks, F.; Trichopoulou, A.; Drescher, G.; Ferro-Luzzi, A.; Helsing, E.; Trichopoulos, D. Mediterranean Diet Pyramid: A Cultural Model for Healthy Eating. *Am J Clin Nutr* 1995, *61*, 1402S-1406S, doi:10.1093/ajcn/61.6.1402S.

142. van den Brandt, P.A.; Schulpen, M. Mediterranean Diet Adherence and Risk of Postmenopausal Breast Cancer: Results of a Cohort Study and Meta-Analysis. *Int J Cancer* 2017, *140*, 2220–2231, doi:10.1002/ijc.30654.

143. Klement, R.; Pazienza, V. Impact of Different Types of Diet on Gut Microbiota Profiles and Cancer Prevention and Treatment. *Medicina (B Aires)* 2019, *55*, 84, doi:10.3390/medicina55040084.

144. Pellegrini, M.; Ippolito, M.; Monge, T.; Violi, R.; Cappello, P.; Ferrocino, I.; Cocolin, L.S.; De Francesco, A.; Bo, S.; Finocchiaro, C. Gut Microbiota Composition after Diet and Probiotics in Overweight Breast Cancer Survivors: A Randomized Open-Label Pilot Intervention Trial. *Nutrition* 2020, *74*, 110749, doi:10.1016/j.nut.2020.110749.

145. Bagga, D.; Wang, L.; Farias-Eisner, R.; Glaspy, J.A.; Reddy, S.T. Differential Effects of Prostaglandin Derived from ω-6 and ω-3 Polyunsaturated Fatty Acids on COX-2 Expression and IL-6 Secretion. *Proceedings of the National Academy of Sciences* 2003, *100*, 1751–1756, doi:10.1073/pnas.0334211100.

146. Wisniewski, P.J.; Dowden, R.A.; Campbell, S.C. Role of Dietary Lipids in Modulating Inflammation through the Gut Microbiota. *Nutrients* 2019, *11*, 117, doi:10.3390/nu11010117.

147. Donohoe, D.R.; Collins, L.B.; Wali, A.; Bigler, R.; Sun, W.; Bultman, S.J. The Warburg Effect Dictates the Mechanism of Butyrate-Mediated Histone Acetylation and Cell Proliferation. *Mol Cell* 2012, *48*, 612–626, doi:10.1016/j.molcel.2012.08.033.

148. Haro, C.; Garcia-Carpintero, S.; Alcala-Diaz, J.F.; Gomez-Delgado, F.; Delgado-Lista, J.; Perez-Martinez, P.; Rangel Zuñiga, O.A.; Quintana-Navarro, G.M.; Landa, B.B.; Clemente, J.C.; et al. The Gut Microbial Community in Metabolic Syndrome Patients Is Modified by Diet. *J Nutr Biochem* 2016, *27*, 27–31, doi:10.1016/j.jnutbio.2015.08.011.

149. Slavin, J. Fiber and Prebiotics: Mechanisms and Health Benefits. *Nutrients* 2013, *5*, 1417–1435, doi:10.3390/nu5041417.

150. Nicholson, J.K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.; Jia, W.; Pettersson, S. Host-Gut Microbiota Metabolic Interactions. *Science (1979)* 2012, *336*, 1262–1267, doi:10.1126/science.1223813.

151. Di Daniele, N.; Di Renzo, L.; Noce, A.; Iacopino, L.; Ferraro, P.M.; Rizzo, M.; Sarlo, F.; Domino, E.; De Lorenzo, A. Effects of Italian Mediterranean Organic Diet vs. Low-Protein Diet in Nephropathic Patients According to MTHFR Genotypes. *J Nephrol* 2014, *27*, 529–536, doi:10.1007/s40620-014-0067-y.

152. Clarke, S.F.; Murphy, E.F.; O'Sullivan, O.; Lucey, A.J.; Humphreys, M.; Hogan, A.; Hayes, P.; O'Reilly, M.; Jeffery, I.B.; Wood-Martin, R.; et al. Exercise and Associated Dietary Extremes Impact on Gut Microbial Diversity. *Gut* 2014, *63*, 1913–1920, doi:10.1136/gutjnl-2013-306541.

153. Clauss, M.; Gérard, P.; Mosca, A.; Leclerc, M. Interplay Between Exercise and Gut Microbiome in the Context of Human Health and Performance. *Front Nutr* 2021, *8*, doi:10.3389/fnut.2021.637010.

154. Estaki, M.; Pither, J.; Baumeister, P.; Little, J.P.; Gill, S.K.; Ghosh, S.; Ahmadi-Vand, Z.; Marsden, K.R.; Gibson, D.L. Cardiorespiratory Fitness as a Predictor of Intestinal Microbial Diversity and Distinct Metagenomic Functions. *Microbiome* 2016, *4*, 42, doi:10.1186/s40168-016-0189-7.

155. Han, M.; Yang, K.; Yang, P.; Zhong, C.; Chen, C.; Wang, S.; Lu, Q.; Ning, K. Stratification of Athletes' Gut Microbiota: The Multifaceted Hubs Associated with Dietary Factors, Physical Characteristics and Performance. *Gut Microbes* 2020, *12*, 1842991, doi:10.1080/19490976.2020.1842991.

156. Donati Zeppa, S.; Sisti, D.; Amatori, S.; Gervasi, M.; Agostini, D.; Piccoli, G.; Bertuccioli, A.; Rocchi, M.B.L.; Stocchi, V.; Sestili, P. High-Intensity Interval Training Promotes the Shift to a Health-Supporting Dietary Pattern in Young Adults. *Nutrients* 2020, *12*, 843, doi:10.3390/nu12030843.

157. Donati Zeppa, S.; Amatori, S.; Sisti, D.; Gervasi, M.; Agostini, D.; Piccoli, G.; Pazienza, V.; Gobbi, P.; Rocchi, M.B.L.; Sestili, P.; et al. Nine Weeks of High-Intensity Indoor Cycling Training Induced Changes in the Microbiota Composition in Non-Athlete Healthy Male College Students. *J Int Soc Sports Nutr* 2021, *18*, 74, doi:10.1186/s12970-021-00471-z.

158. Cheng, R.; Wang, L.; Le, S.; Yang, Y.; Zhao, C.; Zhang, X.; Yang, X.; Xu, T.; Xu, L.; Wiklund, P.; et al. A Randomized Controlled Trial for Response of Microbiome Network to Exercise and Diet Intervention in Patients with Nonalcoholic Fatty Liver Disease. *Nat Commun* 2022, *13*, 2555, doi:10.1038/s41467-022-29968-0.

159. Furber, M.J.W.; Young, G.R.; Holt, G.S.; Pyle, S.; Davison, G.; Roberts, M.G.; Roberts, J.D.; Howatson, G.; Smith, D.L. Gut Microbial Stability Is Associated with Greater Endurance Performance in Athletes Undertaking Dietary Periodization. *mSystems* 2022, *7*, doi:10.1128/msystems.00129-22.

160. Vance, V.; Mourtzakis, M.; McCargar, L.; Hanning, R. Weight Gain in Breast Cancer Survivors: Prevalence, Pattern and Health Consequences. *Obesity Reviews* 2011, *12*, 282–294, doi:10.1111/j.1467-789X.2010.00805.x.

161. Sheflin, A.M.; Whitney, A.K.; Weir, T.L. Cancer-Promoting Effects of Microbial Dysbiosis. *Curr Oncol Rep* 2014, *16*, 406, doi:10.1007/s11912-014-0406-0.

162. Jones, L.W.; Courneya, K.S.; Mackey, J.R.; Muss, H.B.; Pituskin, E.N.; Scott, J.M.; Hornsby, W.E.; Coan, A.D.; Herndon, J.E.; Douglas, P.S.; et al. Cardiopulmonary Function and Age-Related Decline Across the Breast Cancer Survivorship Continuum. *Journal of Clinical Oncology* 2012, *30*, 2530–2537, doi:10.1200/JCO.2011.39.9014.

163. Paulsen, J.A.; Ptacek, T.S.; Carter, S.J.; Liu, N.; Kumar, R.; Hyndman, L.; Lefkowitz, E.J.; Morrow, C.D.; Rogers, L.Q. Gut Microbiota Composition Associated with Alterations in Cardiorespiratory Fitness and Psychosocial Outcomes among Breast Cancer Survivors. *Supportive Care in Cancer* 2017, *25*, 1563–1570, doi:10.1007/s00520-016-3568-5.

164. Di Renzo, L.; Gualtieri, P.; Pivari, F.; Soldati, L.; Attinà, A.; Cinelli, G.; Leggeri, C.; Caparello, G.; Barrea, L.; Scerbo, F.; et al. Eating Habits and Lifestyle Changes during COVID-19 Lockdown: An Italian Survey. *J Transl Med* 2020, *18*, 229, doi:10.1186/s12967-020-02399-5.

165. Montemurro, N. The Emotional Impact of COVID-19: From Medical Staff to Common People. *Brain Behav Immun* 2020, *87*, 23–24, doi:10.1016/j.bbi.2020.03.032.

166. Bennett, G.; Young, E.; Butler, I.; Coe, S. The Impact of Lockdown During the COVID-19 Outbreak on Dietary Habits in Various Population Groups: A Scoping Review. *Front Nutr* 2021, *8*, doi:10.3389/fnut.2021.626432.

167. Natalucci, V.; Villarini, M.; Emili, R.; Acito, M.; Vallorani, L.; Barbieri, E.; Villarini, A. Special Attention to Physical Activity in Breast Cancer Patients during the First Wave of COVID-19 Pandemic in Italy: The DianaWeb Cohort. *J Pers Med* 2021, *11*, 381, doi:10.3390/jpm11050381.

168. Natalucci, V.; Marini, C.F.; Flori, M.; Pietropaolo, F.; Lucertini, F.; Annibalini, G.; Vallorani, L.; Sisti, D.; Saltarelli, R.; Villarini, A.; et al. Effects of a Home-Based Lifestyle Intervention Program on Cardiometabolic Health in Breast Cancer Survivors during the COVID-19 Lockdown. *J Clin Med* 2021, *10*, 2678, doi:10.3390/jcm10122678.

169. FERRI MARINI, C.; SISTI, D.; LEON, A.S.; SKINNER, J.S.; SARZYNSKI, M.A.; BOUCHARD, C.; ROCCHI, M.B.L.; PICCOLI, G.; STOCCHI, V.; FEDERICI, A.; et al. HRR and V˙O2R Fractions Are Not Equivalent: Is It Time to Rethink Aerobic Exercise Prescription Methods? *Med Sci Sports Exerc* 2021, *53*, 174–182, doi:10.1249/MSS.0000000000002434.

170. Ferri Marini, C.; Sisti, D.; Skinner, J.S.; Sarzynski, M.A.; Bouchard, C.; Amatori, S.; Rocchi, M.B.L.; Piccoli, G.; Stocchi, V.; Federici, A.; et al. Effect of Individual Characteristics and Aerobic Training on the %HRR-% *V̇* O 2 R Relationship. *Eur J Sport Sci* 2023, *23*, 1600–1611, doi:10.1080/17461391.2022.2113441.

171. Ferri Marini, C.; Federici, A.; Skinner, J.S.; Piccoli, G.; Stocchi, V.; Zoffoli, L.; Correale, L.; Dell'Anna, S.; Naldini, C.A.; Vandoni, M.; et al. Effect of Steady-State Aerobic Exercise Intensity and Duration on the Relationship between Reserves of Heart Rate and Oxygen Uptake. *PeerJ* 2022, *10*, e13190, doi:10.7717/peerj.13190.

172. CAMPBELL, K.L.; WINTERS-STONE, K.M.; WISKEMANN, J.; MAY, A.M.; SCHWARTZ, A.L.; COURNEYA, K.S.; ZUCKER, D.S.; MATTHEWS, C.E.; LIGIBEL, J.A.; GERBER, L.H.; et al. Exercise Guidelines for Cancer Survivors: Consensus Statement from International Multidisciplinary Roundtable. *Med Sci Sports Exerc* 2019, *51*, 2375–2390, doi:10.1249/MSS.0000000000002116.

173. Xia Y, S.J.C.D.-G. *Statistical Analysis of Microbiome Data with R. Singapore: Springer*; 2018;

174. De Cicco, P.; Catani, M.V.; Gasperi, V.; Sibilano, M.; Quaglietta, M.; Savini, I. Nutrition and Breast Cancer: A Literature Review on Prevention, Treatment and Recurrence. *Nutrients* 2019, *11*, 1514, doi:10.3390/nu11071514.

175. Dieli-Conwright, C.; Orozco, B. Exercise after Breast Cancer Treatment: Current Perspectives. *Breast Cancer: Targets and Therapy* 2015, 353, doi:10.2147/BCTT.S82039.

176. Rock, C.L.; Doyle, C.; Demark-Wahnefried, W.; Meyerhardt, J.; Courneya, K.S.; Schwartz, A.L.; Bandera, E. V.; Hamilton, K.K.; Grant, B.; McCullough, M.; et al. Nutrition and Physical Activity Guidelines for Cancer Survivors. *CA Cancer J Clin* 2012, *62*, 242–274, doi:10.3322/caac.21142.

177. Schmidt, M.E.; Wiskemann, J.; Armbrust, P.; Schneeweiss, A.; Ulrich, C.M.; Steindorf, K. Effects of Resistance Exercise on Fatigue and Quality of Life in Breast Cancer Patients Undergoing Adjuvant Chemotherapy: A Randomized Controlled Trial. *Int J Cancer* 2015, *137*, 471–480, doi:10.1002/ijc.29383.

178. Schmitz, K.H.; Courneya, K.S.; Matthews, C.; Demark-Wahnefried, W.; GALVÃO, D.A.; Pinto, B.M.; IRWIN, M.L.; WOLIN, K.Y.; SEGAL, R.J.; LUCIA, A.; et al. American College of Sports Medicine Roundtable on Exercise Guidelines for Cancer Survivors. *Med Sci Sports Exerc* 2010, *42*, 1409–1426, doi:10.1249/MSS.0b013e3181e0c112.

179. Bruno, E.; Gargano, G.; Villarini, A.; Traina, A.; Johansson, H.; Mano, M.P.; Santucci De Magistris, M.; Simeoni, M.; Consolaro, E.; Mercandino, A.; et al. Adherence to WCRF/AICR Cancer Prevention Recommendations and Metabolic Syndrome in Breast Cancer Patients. *Int J Cancer* 2016, *138*, 237–244, doi:10.1002/ijc.29689.

180. Hojman, P. Exercise Protects from Cancer through Regulation of Immune Function and Inflammation. *Biochem Soc Trans* 2017, *45*, 905–911, doi:10.1042/BST20160466.

181. Hojman, P.; Gehl, J.; Christensen, J.F.; Pedersen, B.K. Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. *Cell Metab* 2018, *27*, 10–21, doi:10.1016/j.cmet.2017.09.015.

182. Campaniello, D.; Corbo, M.R.; Sinigaglia, M.; Speranza, B.; Racioppo, A.; Altieri, C.; Bevilacqua, A. How Diet and Physical Activity Modulate Gut Microbiota: Evidence, and Perspectives. *Nutrients* 2022, *14*, 2456, doi:10.3390/nu14122456.

183. Monda, V.; Villano, I.; Messina, A.; Valenzano, A.; Esposito, T.; Moscatelli, F.; Viggiano, A.; Cibelli, G.; Chieffi, S.; Monda, M.; et al. Exercise Modifies the Gut Microbiota with Positive Health Effects. *Oxid Med Cell Longev* 2017, *2017*, doi:10.1155/2017/3831972.

184. Donati Zeppa, S.; Amatori, S.; Sisti, D.; Gervasi, M.; Agostini, D.; Piccoli, G.; Pazienza, V.; Gobbi, P.; Rocchi, M.B.L.; Sestili, P.; et al. Nine Weeks of High-Intensity Indoor Cycling Training Induced Changes in the Microbiota Composition in Non-Athlete Healthy Male College Students. *J Int Soc Sports Nutr* 2021, *18*, 74, doi:10.1186/s12970-021-00471-z.

185. Parida, S.; Sharma, D. The Microbiome and Cancer: Creating Friendly Neighborhoods and Removing the Foes Within. *Cancer Res* 2021, *81*, 790–800, doi:10.1158/0008-5472.CAN-20-2629.

186. Fernández, M.F.; Reina-Pérez, I.; Astorga, J.M.; Rodríguez-Carrillo, A.; Plaza-Díaz, J.; Fontana, L. Breast Cancer and Its Relationship with the Microbiota. *Int J Environ Res Public Health* 2018, *15*, 1747, doi:10.3390/ijerph15081747.

187. Parada Venegas, D.; De la Fuente, M.K.; Landskron, G.; González, M.J.; Quera, R.; Dijkstra, G.; Harmsen, H.J.M.; Faber, K.N.; Hermoso, M.A. Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front Immunol* 2019, *10*, doi:10.3389/fimmu.2019.00277.

188. Durk, R.P.; Castillo, E.; Márquez-Magaña, L.; Grosicki, G.J.; Bolter, N.D.; Lee, C.M.; Bagley, J.R. Gut Microbiota Composition Is Related to Cardiorespiratory Fitness in Healthy Young Adults. *Int J Sport Nutr Exerc Metab* 2019, *29*, 249–253, doi:10.1123/ijsnem.2018-0024.

189. O'Callaghan, A.; van Sinderen, D. Bifidobacteria and Their Role as Members of the Human Gut Microbiota. *Front Microbiol* 2016, *7*, doi:10.3389/fmicb.2016.00925.

190. Mendoza, L. Potential Effect of Probiotics in the Treatment of Breast Cancer. *Oncol Rev* 2019, *13*, doi:10.4081/oncol.2019.422.

191. Hou, M.-F.; Ou-Yang, F.; Li, C.-L.; Chen, F.-M.; Chuang, C.-H.; Kan, J.-Y.; Wu, C.-C.; Shih, S.-L.; Shiau, J.-P.; Kao, L.-C.; et al. Comprehensive Profiles and Diagnostic Value of Menopausal-Specific Gut Microbiota in Premenopausal Breast Cancer. *Exp Mol Med* 2021, *53*, 1636–1646, doi:10.1038/s12276-021-00686-9.

192. Beam, A.; Clinger, E.; Hao, L. Effect of Diet and Dietary Components on the Composition of the Gut Microbiota. *Nutrients* 2021, *13*, 2795, doi:10.3390/nu13082795.

193. Garcia-Mantrana, I.; Selma-Royo, M.; Alcantara, C.; Collado, M.C. Shifts on Gut Microbiota Associated to Mediterranean Diet Adherence and Specific Dietary Intakes on General Adult Population. *Front Microbiol* 2018, *9*, doi:10.3389/fmicb.2018.00890.

194. Bagheri, S.; Zolghadri, S.; Stanek, A. Beneficial Effects of Anti-Inflammatory Diet in Modulating Gut Microbiota and Controlling Obesity. *Nutrients* 2022, *14*, 3985, doi:10.3390/nu14193985.

195. Gantenbein, K. V.; Kanaka-Gantenbein, C. Mediterranean Diet as an Antioxidant: The Impact on Metabolic Health and Overall Wellbeing. *Nutrients* 2021, *13*, 1951, doi:10.3390/nu13061951.

196. Martín-Peláez, S.; Fito, M.; Castaner, O. Mediterranean Diet Effects on Type 2 Diabetes Prevention, Disease Progression, and Related Mechanisms. A Review. *Nutrients* 2020, *12*, 2236, doi:10.3390/nu12082236.

197. Arora, T.; Tremaroli, V. Therapeutic Potential of Butyrate for Treatment of Type 2 Diabetes. *Front Endocrinol (Lausanne)* 2021, *12*, doi:10.3389/fendo.2021.761834.

198. Gao, Z.; Yin, J.; Zhang, J.; Ward, R.E.; Martin, R.J.; Lefevre, M.; Cefalu, W.T.; Ye, J. Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. *Diabetes* 2009, *58*, 1509–1517, doi:10.2337/db08-1637.

199. Magzal, F.; Shochat, T.; Haimov, I.; Tamir, S.; Asraf, K.; Tuchner-Arieli, M.; Even, C.; Agmon, M. Increased Physical Activity Improves Gut Microbiota Composition and Reduces Short-Chain Fatty Acid Concentrations in Older Adults with Insomnia. *Sci Rep* 2022, *12*, 2265, doi:10.1038/s41598-022-05099-w.

200. Yang, Y.; Du, L.; Shi, D.; Kong, C.; Liu, J.; Liu, G.; Li, X.; Ma, Y. Dysbiosis of Human Gut Microbiome in Young-Onset Colorectal Cancer. *Nat Commun* 2021, *12*, 6757, doi:10.1038/s41467-021-27112-y.

201. Bou Zerdan, M.; Kassab, J.; Meouchy, P.; Haroun, E.; Nehme, R.; Bou Zerdan, M.; Fahed, G.; Petrosino, M.; Dutta, D.; Graziano, S. The Lung Microbiota and Lung Cancer: A Growing Relationship. *Cancers (Basel)* 2022, *14*, 4813, doi:10.3390/cancers14194813.

202. Natalini, J.G.; Singh, S.; Segal, L.N. The Dynamic Lung Microbiome in Health and Disease. *Nat Rev Microbiol* 2023, *21*, 222–235, doi:10.1038/s41579-022-00821-x.

203. Huffnagle, G.B.; Dickson, R.P.; Lukacs, N.W. The Respiratory Tract Microbiome and Lung Inflammation: A Two-Way Street. *Mucosal Immunol* 2017, *10*, 299–306, doi:10.1038/mi.2016.108.

204. Ramesh, V.; Gollavilli, P.N.; Pinna, L.; Siddiqui, M.A.; Turtos, A.M.; Napoli, F.; Antonelli, Y.; Leal-Egaña, A.; Havelund, J.F.; Jakobsen, S.T.; et al. Propionate Reinforces Epithelial Identity and Reduces Aggressiveness of Lung Carcinoma. *EMBO Mol Med* 2023, *15*, doi:10.15252/emmm.202317836.

205. Hendriks, L.E.L.; Remon, J.; Faivre-Finn, C.; Garassino, M.C.; Heymach, J. V.; Kerr, K.M.; Tan, D.S.W.; Veronesi, G.; Reck, M. Non-Small-Cell Lung Cancer. *Nat Rev Dis Primers* 2024, *10*, 71, doi:10.1038/s41572-024-00551-9.

206. Bray, F.; Laversanne, M.; Sung, H.; Ferlay, J.; Siegel, R.L.; Soerjomataram, I.; Jemal, A. Global Cancer Statistics 2022: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2024, *74*, 229–263, doi:10.3322/caac.21834.

207. Jin, C.; Lagoudas, G.K.; Zhao, C.; Bullman, S.; Bhutkar, A.; Hu, B.; Ameh, S.; Sandel, D.; Liang, X.S.; Mazzilli, S.; et al. Commensal Microbiota Promote Lung Cancer Development via Γδ T Cells. *Cell* 2019, *176*, 998-1013.e16, doi:10.1016/j.cell.2018.12.040.

208. McLean, A.E.B.; Kao, S.C.; Barnes, D.J.; Wong, K.K.H.; Scolyer, R.A.; Cooper, W.A.; Kohonen-Corish, M.R.J. The Emerging Role of the Lung Microbiome and Its Importance in Non-Small

Cell Lung Cancer Diagnosis and Treatment. *Lung Cancer* 2022, *165*, 124–132, doi:10.1016/j.lungcan.2022.01.011.

209. Souza, V.G.P.; Forder, A.; Pewarchuk, M.E.; Telkar, N.; de Araujo, R.P.; Stewart, G.L.; Vieira, J.; Reis, P.P.; Lam, W.L. The Complex Role of the Microbiome in Non-Small Cell Lung Cancer Development and Progression. *Cells* 2023, *12*, 2801, doi:10.3390/cells12242801.

210. Al-Qadami, G.H.; Secombe, K.R.; Subramaniam, C.B.; Wardill, H.R.; Bowen, J.M. Gut Microbiota-Derived Short-Chain Fatty Acids: Impact on Cancer Treatment Response and Toxicities. *Microorganisms* 2022, *10*, 2048, doi:10.3390/microorganisms10102048.

211. De Vos, P.; Garrity, G.M.; Jones, D.; Krieg, N.R.; Ludwig, W.; Rainey, F.A.; Schleifer, K.-H.; Whitman, W.B.; Parte, A.C.; Goodfellow, M.; et al. BERGEY'S MANUAL ® OF The Firmicutes WITH CONTRIBUTIONS FROM 165 COLLEAGUES. 2009, doi:10.1007/b92997.

212. Rout-Pitt, N.; Farrow, N.; Parsons, D.; Donnelley, M. Epithelial Mesenchymal Transition (EMT): A Universal Process in Lung Diseases with Implications for Cystic Fibrosis Pathophysiology. *Respir Res* 2018, *19*, 136, doi:10.1186/s12931-018-0834-8.

213. Sun, N.-Y.; Yang, M.-H. Metabolic Reprogramming and Epithelial-Mesenchymal Plasticity: Opportunities and Challenges for Cancer Therapy. *Front Oncol* 2020, *10*, doi:10.3389/fonc.2020.00792.

214. Thomas, S.P.; Denu, J.M. Short-Chain Fatty Acids Activate Acetyltransferase P300. *Elife* 2021, *10*, doi:10.7554/eLife.72171.

215. Larsen, J.E.; Nathan, V.; Osborne, J.K.; Farrow, R.K.; Deb, D.; Sullivan, J.P.; Dospoy, P.D.; Augustyn, A.; Hight, S.K.; Sato, M.; et al. ZEB1 Drives Epithelial-to-Mesenchymal Transition in Lung Cancer. *Journal of Clinical Investigation* 2016, *126*, 3219–3235, doi:10.1172/JCI76725.

216. Brabletz, S.; Schuhwerk, H.; Brabletz, T.; Stemmler, M.P. Dynamic EMT: A Multi-tool for Tumor Progression. *EMBO J* 2021, *40*, doi:10.15252/embj.2021108647.

217. Botticelli, A.; Vernocchi, P.; Marini, F.; Quagliariello, A.; Cerbelli, B.; Reddel, S.; Del Chierico, F.; Di Pietro, F.; Giusti, R.; Tomassini, A.; et al. Gut Metabolomics Profiling of Non-Small Cell Lung Cancer (NSCLC) Patients under Immunotherapy Treatment. *J Transl Med* 2020, *18*, 49, doi:10.1186/s12967-020-02231-0.

218. Kobayashi, M.; Mikami, D.; Uwada, J.; Yazawa, T.; Kamiyama, K.; Kimura, H.; Taniguchi, T.; Iwano, M. A Short-Chain Fatty Acid, Propionate, Enhances the Cytotoxic Effect of Cisplatin by Modulating GPR41 Signaling Pathways in HepG2 Cells. *Oncotarget* 2018, *9*, 31342–31354, doi:10.18632/oncotarget.25809.

219. Ghorbani, P.; Santhakumar, P.; Hu, Q.; Djiadeu, P.; Wolever, T.M.S.; Palaniyar, N.; Grasemann, H. Short-Chain Fatty Acids Affect Cystic Fibrosis Airway Inflammation and Bacterial Growth. *European Respiratory Journal* 2015, *46*, 1033–1045, doi:10.1183/09031936.00143614.

220. Dalile, B.; Van Oudenhove, L.; Vervliet, B.; Verbeke, K. The Role of Short-Chain Fatty Acids in Microbiota–Gut–Brain Communication. *Nat Rev Gastroenterol Hepatol* 2019, *16*, 461–478, doi:10.1038/s41575-019-0157-3.

221. Mirzaei, R.; Afaghi, A.; Babakhani, S.; Sohrabi, M.R.; Hosseini-Fard, S.R.; Babolhavaeji, K.; Khani Ali Akbari, S.; Yousefimashouf, R.; Karampoor, S. Role of Microbiota-Derived Short-Chain Fatty Acids in Cancer Development and Prevention. *Biomedicine & Pharmacotherapy* 2021, *139*, 111619, doi:10.1016/j.biopha.2021.111619.

Abbreviations

AA: Acetic acid

AADC: Aromatic amino acid decarboxylase

AMPK: AMP-activated protein kinase

ASD: Autism Spectrum Disorder

ASD: Autism Spectrum Disorder

BBB: Blood–brain barrier

BC: Breast cancer

BSH: Bile salt hydrolase

CDI: *Clostridioides difficile* infection

CNS: Central nervous system

CRC: Colorectal Cancer

C-section: cesarean section

DA: dopaminergic

DAT: Dopamine transporter

DRP1: Dynamin-Related Protein 1

EECs: Enteroendocrine cells

ENS: Effective number of species

ENS: Enteric nervous systems

ERK: Extracellular Signal-Regulated Kinase

FDR: False discovery rate

FFAR2: Free Fatty Acid Receptor 2

FMT: Fecal microbiota transplantation

GIT: Gastrointestinal tract

GLP-1: Glucagon-like peptide-1

GLP-1Ras: GLP-1 receptor agonists

GM: Gut microbiota

H&Y: Hoehn and Yahr

H3K27ac: p300-mediated histone H3 lysine 27 acetylation

HOMA: Homeostatic Model Assessment

HPV-16: Human Papillomavirus type 16

IBD: Inflammatory Bowel Disease

IBS: Irritable Bowel Syndrome

IgA: Immunoglobulin A

IL-12: Interleukin-12

IL-17: Interleukin-17

IL-18: interleukin-18

IL-22: Interleukin-22

IL-4: interleukin-4

IL-6: Interleukin-6

IL-8: Interleukin-8

iRBD: REM sleep behavior disorder

KRAS: Kirsten Rat Sarcoma Viral Oncogene Homolog

LBs: Lewy bodies

LEDD: Levodopa Equivalent Daily Dose

LGR5: Leucine-rich repeat-containing G protein-coupled receptor 5

LPS: lipopolysaccharide

M: Microfold

MAMPs: microbe-associated molecular patterns

MDS-UPDRS III: Movement Disorder Society–Unified Parkinson's Disease Rating Scale Part III

MGO: Methylglyoxal

MHI: Microbiome Health Index

MUC2: Mucin-2

MUC5AC: Mucin-5AC

NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells

NLRs: NOD-like receptors

nMDS: Non-metric dimensional scaling

NSCLC: Non-small cell lung cancer

OLS: Ordinary least squares

OPA1: Optic Atrophy 1

OTUs: Operational Taxonomic Units

p53: Tumor Protein p53

PA: propionic acid

PCoA: Principal Coordinates Analysis

PD: Parkinson's Disease

PERMANOVA: Permutational Multivariate Analysis of Variance

PI3K: Phosphoinositide 3-Kinase

PYY: Peptide YY

RA: Reumatoid Arthritis

RM-MANOVA: Multivariate analysis of variance for repeated measures

SCFA: short-chain fatty acid

SCLC: Small-cell lung cancer

SLE: Systemic Lupus Erythematosus

SNARE: Soluble N-ethylmaleimide–Sensitive Factor Attachment Protein Receptor

TLRs: Toll-like receptors

TyrDC: Tyrosine decarboxylase

VEGF: Vascular Endothelial Growth Factor

Publications included in the thesis

Donati Zeppa, S.; Natalucci, V.; Agostini, D.; Vallorani, L.; Amatori, S.; Sisti, D.; Rocchi, M.B.L.; Pazienza, V.; Perri, F.; Villani, A.; et al. Changes in Gut Microbiota Composition after 12 Weeks of a Home Based Lifestyle Intervention in Breast Cancer Survivors during the COVID-19 Lockdown. *Front. Oncol.* ***2023****, 13,* 1225645, doi:10.3389/fonc.2023.1225645.