Annals of Gastroenterology (2026) **39,** 191-201

Determinants of the healthy gut microbiome: core features, modifying factors and normal functions

Ilektra Kyrochristou[a,b], Gerasimia Kyrochristou[a], Fotios Fousekis[c], Konstantinos Katsanos[c], Dimitrios Schizas[d], Konstantinos Vlachos[a], Georgios D. Lianos[a]

University Hospital of Ioannina, Ioannina; General Hospital of Nikaia and Piraeus, Athens; National and Kapodistrian University of Athens, Greece

Abstract

The human gut microbiome represents a complex and dynamic ecosystem that is central to maintaining health and preventing disease. Defining a "normal" gut microbiome remains challenging, given the significant variability arising from host physiology, lifestyle, genetics, geography and environmental exposures. This review synthesizes current evidence regarding the composition and functions of the gut microbiota in healthy individuals from diverse populations. At the taxonomic level, healthy gut microbial communities are typically dominated by the phyla *Firmicutes* and *Bacteroidetes*, with additional contributions from *Actinobacteria* and *Proteobacteria*. However, substantial inter-individual and regional differences are observed, such as a higher prevalence of *Prevotella* in populations consuming fiber-rich Eastern diets, and greater *Bacteroides* abundance in Western cohorts. Anatomical location and health status also influence alpha-diversity, underscoring the need to interpret diversity metrics within context. Furthermore, the gut microbiome performs essential functional roles across multiple organ systems, including fermentation of dietary fibers into short-chain fatty acids, regulation of immune responses, modulation of the gut-brain axis, maintenance of intestinal barrier integrity, and support of cardiovascular and hepatic functions. These findings support the conceptualization of the microbiome as a multifunctional organ system that integrates host and environmental signals. In summary, a healthy gut microbiome is best understood as a dynamic equilibrium, characterized by functional resilience and adaptability, rather than a fixed microbial profile. Interpreting this variability is crucial for developing targeted interventions to prevent disease.

Keywords Microbiome, alpha-diversity, gut microbiota, healthy, normal gut

Ann Gastroenterol 2026; 39 (2): 191-201

[a]Department of Surgery, University Hospital of Ioannina, Ioannina (Ilektra Kyrochristou, Gerasimia Kyrochristou, Konstantinos Vlachos, Georgios D. Lianos); [b]Department of Emergency Medicine, General Hospital of Nikaia and Piraeus, Athens (Ilektra Kyrochristou); [c]Department of Gastroenterology, University Hospital of Ioannina, Ioannina (Fotios Fousekis Konstantinos Katsanos); [d]1st Department of Surgery, National and Kapodistrian University of Athens (Dimitrios Schizas), Greece

Conflict of Interest: None

Correspondence to: Ilektra Kyrochristou, MD, MSc, Department of Surgery, University Hospital of Ioannina, Ioannina; Department of Emergency Medicine, General Hospital of Nikaia and Piraeus, Dimitriou Mantouvalou 3, 18561 Athens, Greece, e-mail: electra.cyro@gmail.com

Received 15 October 2025; accepted 6 December 2025; published online 26 January 2026

DOI: https://doi.org/10.20524/aog.2026.1034

Introduction

The vast and diverse microbial community that colonizes our gastrointestinal tract is collectively referred to as the gut microbiome. All these bacteria, archaea, viruses and fungi [1] comprise an entire ecosystem increasingly recognized as a principal regulator of host physiology, playing essential roles in nutrient metabolism, maintaining epithelial integrity and modulating immune responses [2]. A balanced and functionally diverse microbiome is considered fundamental to human health. However, defining a "normal" gut microbiome poses a challenge, given the considerable variability among individuals that is influenced by diet, age, genetics, geography and environmental exposures.

A healthy human gut microbiome contains approximately 100 trillion microbes. The types and numbers of these microorganisms vary throughout the gastrointestinal tract, as a result of different pH levels and host secretions [3].

In addition to internal genetic and physiological traits, external factors, such as antibiotic use, lifestyle, diet, stress,

aging, and diseases, can significantly alter the composition and diversity of the gut microbiota. These external forces shape the balance of bacteria in the gut, influencing both gut health and overall well-being [4].

All the above intrinsic and extrinsic factors highlight the challenge of defining a normal gut microbiome, especially when the concept of the "healthy individual" is taken into consideration. The term "healthy" is, in fact, very personalized, even if it does not seem so, because apart from the absence of any diagnosed disease, each person's healthy state refers to different standards in terms of physical status and behavioral habits, such as sleep or mood.

Furthermore, microbiome research raises several points open to interpretation. To begin with, as research methods, data collection and analysis are not standardized among researchers, inconsistencies in findings are always present. Additionally, the functional aspects of the microbiome, beyond simple composition, are complex, as the presence of a gene does not guarantee its function, and the relationship between dysbiosis (an altered microbiome) and disease is not always clear [5].

The current review presents literature findings on the composition of a supposedly healthy gut microbiome, based on data from diverse geographical areas. In addition, it presents factors that influence the shape of a normal microbiome throughout human life. Lastly, it demonstrates the role of several microbes normally found in the intestinal flora in various normal functions of different organ systems, such as the immune system, the nervous system and the gastrointestinal system.

Prior reviews, such as those by Van Hul *et al* [1] and McBurney *et al* [5], have emphasized the conceptual frameworks and the regulatory perspectives of the normal microbiome. In contrast, the present review integrates global taxonomic data, host- and lifestyle-dependent modifiers, and multi-system functional roles of the microbiome. The goal of the writers was, via a combined perspective, to better delineate the range of microbial states compatible with health.

The normal gut microbiome - composition in healthy individuals

A structured search was performed in PubMed, Embase, and Google Scholar using the terms 'healthy', 'gut microbiome', 'microbiota composition', and 'adults'. Studies published between January 2000 and December 2024 were considered. Inclusion criteria were: (1) adults ≥18 years; (2) clearly defined healthy population; (3) taxonomic data based on 16S rRNA or metagenomics; (4) English language. Exclusion criteria included recent antibiotic use, chronic disease, pregnancy, or incomplete methodological description. After a thorough literature review and using the snowball technique, 13 original research articles containing information on the gut microbiota of healthy individuals worldwide were identified [6-18].

As considerable methodological heterogeneity and variable risk of bias were detected after a critical appraisal of the included studies, most were classified as moderate risk using the ROBINS-2 tool, with several rated as having serious risk due to small sample sizes, limited dietary or medication controls, and cross-sectional designs. Another obstacle to direct comparison across studies was the inconsistent sequencing platforms and DNA extraction methods. Only larger, well-characterized studies [11,18] approached higher methodological rigor, though they too remained observational. These limitations underscore the need for standardized study protocols, copious covariate adjustment, and longitudinal study designs to more reliably define the characteristics of a "normal" gut microbiome. Extended data on the risk of bias are presented in Supplementary Table 1.

In total, 2238 healthy individuals from Asia, America and Northern Europe were included in the qualitative synthesis. Eleven articles provided information on the participants' sex, with 52.7% (1110/2105) being men and 47.3% (995/2105) women. Their ages and body mass indexes varied and are presented in Table 1.

These articles give a perception of what "healthy" looks like, and make it very apparent how tricky this phrase can be. Most researchers consider as "healthy", the microbiome of individuals not demonstrating any other illness. This is also depicted in the exclusion criteria of 6 of the studies included in the synthesis (Table 2).

A "healthy" or "normal" gut microbiome does not correspond to a single universal microbial composition; rather, it reflects a state of balance and functionality that supports host physiology. Core features of a healthy microbiome include high microbial diversity, functional redundancy, and relative stability over time, even in the face of external perturbations, such as dietary changes or minor infections [19].

Some large cross-continent studies demonstrate that a healthy gut microbiome varies significantly with age and geographical location, with diet and lifestyle being key influencing factors [11,14,20]. Research shows that microbiome composition changes throughout life, with distinct microbial communities linked to industrialized vs. non-Western diets and further influenced by factors like long-term diet quality or living in a long-term care facility [20,21].

The findings summarized in Table 3 describe the gut microbiome characteristics of individuals living in different geographic areas. It is evident that some commonalities exist, but at the same time one can spot significant regional variations. The predominance of the phyla *Firmicutes* and *Bacteroidetes* highlights them as the core constituents of the healthy gut across populations [6,8-12,14-15,18]. Other taxa, such as *Actinobacteria* and *Proteobacteria*, also emerge consistently across populations, although usually in lower relative abundance [6,10-12,14,18]. These shared features suggest that, despite dietary, environmental and genetic differences, a "baseline" microbial signature of health can be defined at higher taxonomic levels.

Additionally, one can detect marked geographical and population-specific differences, as the relatively high abundances of *Prevotella* [6,8,10-11,13,15] in Estonian and Asian studies, consistent with dietary patterns rich in plant-based carbohydrates and fibers. In contrast, Western

Table 1 Patients' demographics

Researcher [ref.]	Year of research	Population	Number of patients	Men: women	Age (mean±SD) - years	BMI (mean±SD) - kg/m^2
Yu *et al* [6]	2017-2019	Control group of healthy individuals - Chinese	31	11:20	51.32±13.61	24.85±3.49
Shalon *et al* [7]	2022	Healthy individuals - American	15	7:08	42±10.5	
Pihelgas *et al* [8]	2022	Healthy individuals - Estonian	12	2:10	40.5±4.75	22.9 (MEDIAN)
Olivares *et al* [9]	2021	Healthy individuals - Brazilian	18	2:16	34±2.69	21.38±0.70
Xia *et al* [10]	2024	Healthy elderly individuals (>60 years old) - Chinese	10	5:5	62.40±7.34	24.52±2.27
Zhao *et al* [11]	2018	Healthy adolescents - Chinese	302	154:142	10.64±0.81	
Khachroub *et al* [12]	2021	Healthy individuals - Tunisian	19	7:12	29.84±8	21.78±1.63
Zhang *et al* [13]	2021	Healthy individuals - Chinese	17	7:10	19.662±0.611	20.167±2.172
Brooks *et al* [14]	2018	Healthy individuals - Asian-Pacific Islanders [N=88], Caucasians [N=1237], Hispanics [N=37], and African Americans	1375	718:657	40.2±9.7	24±4.7
Ang *et al* [15]	2020-2021	Healthy individuals - White and East Asian	46	not provided	not provided	RANGE 18.5-52
Yasir *et al* [16]	2013-2015	Healthy individuals - French and from Saudi Arabia	29	20:09	31.5±5.25	24.5±3.2
Kulecka *et al* [17]	2018	Healthy individuals (11) and athletes (70)	81	not provided	RANGE 14-72	not provided
Takagi *et al* [18]	2016-2017	Healthy individuals - Japanese	283	177:106	64.2±15	not provided

**BMI, body mass index*

Table 2 Exclusion criteria of research on the normal (healthy) gut microbiome

Researcher [ref.]	Number of patients	Exclusion criteria
Shalon *et al* [7]	15	History of: prior gastric or esophageal surgery, including lap banding or bariatric surgery, bowel obstruction, gastric outlet obstruction, diverticulitis, IBD, ileostomy or colostomy, gastric or esophageal cancer, achalasia, esophageal diverticulum, active dysphagia or odynophagia, or active medication use for any gastrointestinal conditions Pregnancy or planned pregnancy within 30 days of the screening visit or breastfeeding Any form of active substance abuse or dependence, any unstable medical or psychiatric disorder A clinical condition that could potentially pose a health risk to the individual while they were involved in the study
Olivares *et al* [9]	18	Individuals using dietary supplements, prebiotics and/or probiotics during the past 2 months Women who were pregnant or breastfeeding Menopausal women Individuals diagnosed with diabetes mellitus or using hypoglycemic medications Individuals with hepatic insufficiency, inflammatory intestinal diseases or renal insufficiency; individuals using antibiotics during the past 2 months, laxatives, lipid-lowering drugs or corticoid substances; individuals with recent episodes of diarrhea during the past 2 months Vegetarians
Xia *et al* [10]	10	History of metabolic diseases, including diabetes and thyroid disease History of peptic diseases, including intestinal inflammatory ulcers Use of antibiotics, probiotics, prebiotics, postbiotics, or immunosuppressive agents in the previous 2 months
Zhao *et al* [11]	302	Antibiotic treatment for the past 15 days Gastrointestinal dysfunction or previous gastrointestinal disease history Diarrhea, abdominal distension, abdominal pain, or constipation within the past 15 days
Zhang *et al* [13]	17	Suffering from any gastrointestinal disorder, or having recently suffered from severe diarrhea and constipation
Yasir *et al* [16]	29	Individuals aged over 18 years History of colon cancer, inflammatory bowel disease, or acute or chronic diarrhea in the previous 8 weeks Treatment with an antibiotic in the 6 months before fecal sampling.

IBD, inflammatory bowel disease

Table 3 Normal gut microbiome of healthy adults from different geographical areas

Researcher [ref.]	Geographical area	No. of patients	Sample type	Alpha-diversity
Yu *et al* [6]	China	31	feces	1. Lower alpha-diversity than constipated patients 2. Dominant phyla: *Firmicutes*, *Bacteroidetes*, *Proteobacteria*.
Shalon *et al* [7]	USA	15	luminal contents (liquid) and feces	1. Alpha-diversity: Measured using Shannon index and observed OTU counts; both indices showed the highest diversity in the proximal small intestine, with a gradual decline toward distal regions and fecal samples. 2. Main microbes: *Bacteroides, Alistipes, & Bilophila*
Pihelgas *et al* [8]	Estonia	12	feces	Most dominant genus: *Prevotella, Bacteroides, Fusicatenibacter, Christensenellaceae, & Phascolarctobacterium*
Olivares *et al* [9]	Brazil	18	feces	Dominant phyla: *Firmicutes* and related taxonomic levels, class *Bacteroidia*, order *Bacteroidales,* and family *Prevotellaceae*
Xia *et al* [10]	China	10	feces	Dominant phyla: *Firmicutes and Bacteroidetes*, followed by *Desulfobacterota, Campilobacterota, Actinobacteriota, and Deferribacterota.*
Zhao *et al* [11]	China	302	feces	Dominant phylum: *Firmicutes,* followed by Bacteroidota, Actinobacteria, and *Proteobacteria* in healthy adolescents
Khachroub *et al* [12]	Tunisia	19	feces	1. Most abundant phyla: *Firmicutes, Bacteroidota, Actinobacteriota, and Proteobacteria* 2. Most abundant families: *Lachnospiraceae, Ruminococcaceae, Bacteroidaceae, and Prevotellaceae*
Zhang *et al* [13]	China	17	feces	1. Most dominant taxa: *Clostridium ramosum, Eubacterium sp 1_3, Gemella, and Bacillales Insertae Sedis XI*
Brooks *et al* [14]	Asia – Europe – USA - Latin America	1375	*not specified	Across ethnicities, *Firmicutes and Bacteroidetes* each~35–54% of total microbiota; consistent presence of *Proteobacteria, Actinobacteria, Verrucomicrobia*.
Ang *et al* [15]	Asia	46	feces	1. Most abundant genera: *Blautia, Bacteroides, Faecalibacterium, and Agathobacter* 2. East Asians also demonstrate high numbers of *Prevotella genus*
Yasir *et al* [16]	France & Saudi Arabia	29	feces	1. French participants had higher *Verrucomicrobia and Bifidobacterium* 2. *Fusobacteria* and *Lactobacillus sakei* only in French group.
Kulecka *et al* [17]	Poland	81	feces	1. Both athlete groups (marathon runners and skiers) showed: reduced abundance of *Bacteroidetes* (a major gut phylum), and elevated levels of *Prevotella* 2. Marathon runners specifically exhibited: elevated *Haemophilus* and *Veillonella,* and reduced *Blautia* and *Faecalibacterium*
Takagi *et al* [18]	Japan	283	feces	1. Four most dominant phyla*: Firmicutes, Bacteroidetes, Actinobacteria,* and *Proteobacteria* 2. Seven most dominant genera: *Bacteroides, Bifidobacterium, Faecalibacterium, Blautia, Ruminoocccus (family Ruminococcaceae), Roseburia, and Prevotella*

OTU, operational taxonomic unit
**Sample type not consistently specified for each dataset; most cohorts used stool samples*

populations (e.g. the USA and France) that follow diets rich in animal protein and fat demonstrate higher Bacteroides abundance [7,14,16]. French individuals also showed higher levels of *Verrucomicrobia* and *Bifidobacterium* than Saudi participants, underscoring how regional lifestyle, diet, and possibly host genetics, shape gut microbial composition [16]. Such differences provide evidence for the adaptive plasticity of the gut microbiome in response to external factors, while still maintaining core microbial taxa associated with health.

Differences in alpha-diversity further enrich these findings: for example, healthy Chinese participants had lower alpha-diversity than constipated individuals, challenging the assumption that greater diversity is always beneficial [6]. Additionally, throughout the gastrointestinal tract, a significant spatial variation in alpha-diversity is observed, with greater diversity in the small intestine than in distal regions [7]. These observations suggest that alpha diversity must be interpreted contextually, considering anatomical site, health status and ecological balance, rather than being treated as a uniform marker of gut health.

Finally, large-scale, multi-ethnic studies reinforce the notion that, while core taxa remain relatively stable, there is significant heterogeneity in the gut microbiome across populations [14]. The need to move beyond a universal definition of the "normal" microbiome is evident when considering this variability. Instead, a range of healthy microbial profiles shaped by geography, culture and lifestyle should define "healthy". The evidence suggests that gut health is best understood not as

a fixed microbial composition. To better understand it, we should look at it as a flexible equilibrium that balances shared functional capacities with population-specific adaptations.

Thus, taxonomically healthy gut microbial communities are often dominated by members of the phyla *Firmicutes* and *Bacteroidetes*, with contributions from *Actinobacteria* and *Proteobacteria* at lower levels. However, the emphasis of the global research community has shifted from strictly compositional profiles toward the metabolic and functional capacity of these microbes. Key functions provided by a balanced microbiome include the fermentation of dietary fibers into short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate; modulation of the immune system; maintenance of gut epithelial integrity; and competitive exclusion of pathogenic microorganisms [22,23].

Gut health is not only the absence of gastrointestinal disease, but also the optimal functioning of digestive and absorptive processes. Towards this goal, the intact intestinal barrier, effective immune regulation and minimal gastrointestinal discomfort are all of profound significance [24]. A healthy intestine is increasingly recognized as a fundamental factor in systemic health, influencing metabolic, neurological and immunological processes. Disruptions in microbiome composition and function—commonly referred to as dysbiosis—have been associated with conditions ranging from inflammatory bowel disease and obesity to mental health disorders, highlighting the importance of microbial homeostasis [25].

In summary, the concept of a "normal" and "healthy" gut is not defined by a fixed microbial signature. It is rather the dynamic balance, diversity, and resilience of the gut microbiome, along with its ability to sustain host health through key metabolic, immune, and protective functions.

Host, environmental and lifestyle influences on the gut microbiome in healthy adults

The studies summarized in Table 4 reveal both common patterns and notable differences in the gut microbiome of healthy adults. Despite differences across geographic regions, one consistent finding is the predominance of the *Firmicutes* and *Bacteroidetes* phyla, which form the backbone of microbial communities in the human gastrointestinal tract [6,9,18]. These groups are closely linked to key metabolic functions that support host health, such as the production of SCFAs and the maintenance of the gut lining. However, the varying levels of these microbes across different studies suggest that the gut microbiome is not static—it is a flexible ecosystem shaped by factors related to both the host and their environment.

Multiple researchers emphasize the influence of host physiology and health on microbiome composition. For example, individuals with constipation had lower levels of *Bacteroidaceae* and higher levels of *Ruminococcaceae*, whereas individuals with hyperlipidemia showed elevated levels of *Campilobacterota* and *Proteobacteria* [6,10]. Similarly, obesity seems to coexist with a higher proportion of Gram-negative bacteria, suggesting a potential link between metabolic disorders, inflammation, and shifts in the microbial population [9]. These results highlight how sensitive the microbiome is to changes in health, even among people considered generally healthy, and suggest its value as both a marker and a possible contributor to disease risk.

Diet also stands out as a significant factor in shaping the gut microbiome. Data are still limited, but research so far indicates that, while overall microbial diversity was not significantly affected by fiber supplementation, specific groups such as *Bacteroides* and *Prevotella* did change in response to diet, often in ways unique to each individual [8]. Additionally, macronutrient intake in athletes has been linked to the abundance of certain bacteria: *Prevotella* was less common with higher sucrose intake, while *Agathobacter* was more prevalent in those consuming more fiber [17]. These findings demonstrate that the microbiome responds, not just to overall diet quality, but also to particular nutrients, highlighting the importance of personalized nutrition in this field.

The gut microbiome is also influenced by geography and lifestyle. For instance, a study in urban and rural areas of China by Zhao *et al* [11] found significant differences between adolescents living in those areas, with *Bifidobacterium* more common in rural populations and *Bacteroides* more prevalent in urban populations. Across geographical compartments, French participants exhibited greater gut microbial diversity than Saudi participants. This finding was independent of weight, suggesting that cultural and dietary differences shape microbiome diversity [16]. Likewise, clear distinctions between East Asian and White populations, independent of obesity status, were detected, while a subtle yet significant variation across ethnicities was observed in a large multi-ethnic cohort [14,16]. These findings emphasize the role of geography, culture and lifestyle as fundamental determinants of microbial composition.

In addition to diet and geography, sex and gender contribute to microbiome variability. For example, male participants had a higher relative abundance of *Firmicutes* than females, suggesting that sex-specific hormonal and physiological factors may shape microbial communities [12]. Taxonomic differences between population groups that may intersect with both sex and ethnicity were also noted [15]. Taken together, these results suggest that host biological sex interacts with other determinants, such as diet, culture, and environment, producing subtle but measurable differences in microbiota composition.

Sleep and circadian rhythms also seem to influence gut microbial ecology. In a study of 302 individuals, a significant correlation was observed between poor sleep quality and an increased abundance of *Erysipelotrichaceae*, accompanied by a reduction in *Tenericutes* [13]. Microbial beta-diversity was also positively associated with sleep duration among adolescents, with those who slept more than 6 h demonstrating a microbiome of "higher" diversity and quality [11]. These findings support emerging evidence that circadian misalignment and sleep disruption alter host metabolism and immune function, thereby reshaping microbial communities. Such observations extend the scope of microbiome research beyond diet and

Table 4 The gut microbiome of healthy adults and related conditions

Researcher [ref.]	Primary outcome – Main finding	Notable observations
Yu *et al* [6]	Constipation alters the gut microbiota, with decreased *Bacteroidaceae* and increased *Ruminococcaceae.*	Constipation alters the gut microbiota, with reduced *Bacteroidaceae* and elevated *Ruminococcaceae.*
Shalon *et al* [7]	The stool proteome is not fully representative of the intestinal proteome.	Stool and intestinal samples share most vOTUs
Pihelgas *et al* [8]	Gut alpha-diversity remained stable despite increased dietary fiber.	*Bacteroides* type bacteria are more prone to changes in dietary intake
Olivares *et al* [9]	Obesity is associated with microbiome shifts and elevated Gram-negative bacteria.	
Xia *et al* [10]	Hyperlipidemia is linked to greater abundance of *Campilobacterota and Proteobacteria.*	Females show greater abundances of *Parabacteroides*, while men's microbiota is rich *in Bacteroides*.
Zhao *et al* [11]	Urbanization influences adolescent gut microbiota composition.	*Bacteroides* was most found in cities and less commonly in towns and rural areas. There were significant differences in the genus-level bacterial community structure (beta diversity) among adolescents with different sleep durations
Khachroub *et al* [12]	Obesity and sex affect microbial composition.	The relative abundance of *Firmicutes* was significantly greater in males compared to females. Healthy individuals showed a higher proportion of *Proteobacteria* and a lower proportion of *Atopobiaceae* and *Peptostreptococcaceae*.
Zhang *et al* [13]	Poor sleep quality associated with microbiome composition.	Higher abundance of the family *Erysipelotrichaceae (phylum Firmicutes)* in participants with poor sleep quality. The relative abundance of the phylum *Tenericutes* and class *Mollicutes* in subjects with poor sleep quality was lower than in healthy individuals.
Brooks *et al* [14]	Ethnicity, BMI, and sex influenced microbiota composition.	OTUs and evenness (Equitability) significantly vary across ethnicities with the following ranks: Hispanics > Caucasians > Asian-Pacific Islanders > African Americans
Ang *et al* [15]	Microbiota differed between White and East Asian participants, independent of weight.	*Streptococcus and Bacteroides* are significantly more abundant in East Asians (positive fold change). *Clostridia and [Eubacterium]* are more abundant in Whites (negative fold change).
Yasir *et al* [16]	French individuals exhibited greater microbial richness compared to Saudis.	
Kulecka *et al* [17]	Exercise influenced microbiota composition in athletes.	*Prevotella* inversely correlated with sucrose intake. *Phascolarctobacterium* inversely correlated with polyunsaturated fatty acids (PUFAs) intake. *Christensenellaceae* positively correlated with folic acid intake. *Agathobacter* positively correlated with dietary fiber intake
Takagi *et al* [18]	Healthy adults showed higher abundance of *Prevotella.*	Bacteroides linked to IBD; *Ruminococcaceae* linked to cardiovascular/ neurological disease.

IBD, inflammatory bowel disease; OTU, operational taxonomic unit; vOTU, viral operational taxonomic unit; SCFA, short-chain fatty acid; PUFA, polyunsaturated fatty acid; BMI, body mass index

disease, highlighting the importance of behavioral and lifestyle factors.

Another determinant not consistently addressed in the reviewed studies is host genetics. Although the studies summarized here primarily focus on environmental and lifestyle influences, previous research has shown that specific host genotypes can shape microbial composition, particularly for taxa such as *Bifidobacterium* and *Christensenellaceae*. The interaction between host genetic background and external exposures may partially explain the inter-individual variability observed across geographically or culturally similar groups [14]. Similarly, age and developmental stage play a crucial role: while the included studies focused on adults, Zhao *et al* [11] highlighted adolescence as a period of transition, during which factors such as urbanization and lifestyle strongly modulate microbial composition.

Finally, medication use and external exposures represent critical yet underreported factors in many of the included studies. As highlighted by current evidence, antibiotics and dietary fiber can have detrimental effects on gut microbial composition, with sometimes radical effects even among healthy individuals [7]. Apart from antibiotics, other commonly used

drugs, such as proton pump inhibitors, metformin and non-steroidal anti-inflammatory drugs, are known to have varying effects on microbial communities. Environmental exposures, including pollutants, sanitation and early-life microbial colonization, also leave lasting imprints on gut ecology. Their absence from many datasets indicates a gap in current research that must be addressed in future cross-population studies.

The various factors that synthesize a normal gut microbiome are depicted in Fig. 1. In addition, data presented in Table 4 further reinforce the concept of the gut microbiome as both stable and adaptable. While certain phyla, such as *Firmicutes* and *Bacteroidetes*, serve as universal hallmarks of gut health, significant variability arises from physiological states, diet, geography, sex, lifestyle behaviors and external exposures. Factors not explicitly covered in the reviewed studies, such as host genetics, age, medication use and circadian rhythm, further enrich this picture, underscoring the complexity of defining a universal "normal" microbiome. The evidence supports viewing gut health as a dynamic equilibrium, rather than a fixed system, in which microbial composition and function reflect a balance between shared core features and individualized, context-dependent adaptations.

Normal functions of the gut microbiome

The gut microbiome plays diverse and systemic roles in maintaining human health. In a normal microbiome, each microbial element contributes to homeostasis of the gastrointestinal lumen, and across immune, neuroendocrine, hepatic, cardiovascular and metabolic axes. By producing metabolites and activating host signaling pathways, these microorganisms function as an integrated metabolic organ. As summarized in Table 5, many of these effects converge on conserved mechanisms, particularly the production of SCFAs, tryptophan-derived metabolites and neurotransmitter-like compounds—core features of a well-functioning microbial ecosystem.

A normal gut microbiome contributes substantially to immune homeostasis. Laboratory research has found that certain bacteria, like *Peptostreptococcus russellii* and *Lactobacillus*, can transform tryptophan (an amino acid found in many foods)

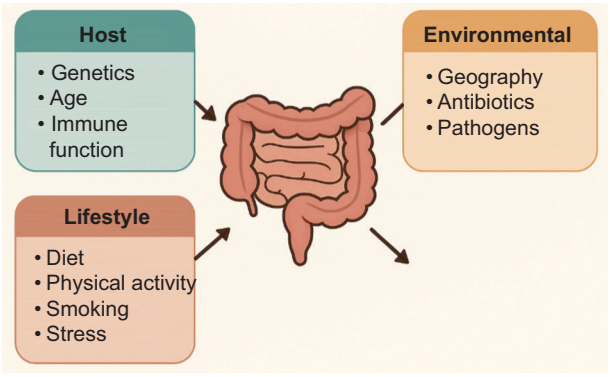

Figure 1 Key host, environmental and lifestyle determinants of the healthy gut microbiome

into molecules that activate the aryl hydrocarbon receptor. This process helps guide the development of immune cells, the release of signaling molecules called cytokines, and the strength of the gut's protective lining [26,27]. Studies in people also suggest that some byproducts made by these bacteria, such as indole-3-propionic acid (IPA) and 4-hydroxyphenylpropionic acid (4-OH-PPA), support a healthy immune response [28,29]. Together, these findings show that when the microbiome is working well, it helps regulate our immune defenses, while disruptions in these pathways are often seen in people with gut imbalances and inflammatory diseases.

The normal microbiome participates directly in gut-brain communication via neural, immune and endocrine pathways. Multiple studies, combining animal models and human clinical research, demonstrate that taxa such as *Bacteroides*, *Lactobacillus*, *Bifidobacterium* and *Ruminococcus* produce neurotransmitter precursors (e.g., serotonin from tryptophan), induce ghrelin secretion, or modulate GABA receptor activity [30-38]. Butyrate-producing bacteria, characteristic of a healthy adult microbiome, have been associated with improved cognitive performance and reduced depressive symptoms in both preclinical and human studies.

The gut-liver axis is yet another paradigm of the systemic functions of a normal microbiome. Human data show that *Lactobacillus* spp. increase intestinal-derived HDL3, reduce hepatic endotoxin exposure, and limit macrophage activation [39,40]. SCFAs produced by *Clostridium* and *Bifidobacterium* species similarly modulate hepatic immune responses, an observation primarily based on animal data [41]. All the above imply a protective role of specific bacteria against liver inflammation, fibrosis, and metabolic disturbances.

Cardiovascular regulation constitutes another system that is influenced in various ways. Other researchers report, using both animal and human evidence, that members of *Firmicutes* and *Lachnospiraceae* produce SCFAs that contribute to blood pressure regulation and the control of inflammation [42-44]. *Lactobacillus* and *Bifidobacterium* are proposed to potentially lower serum cholesterol and improve vascular function. *Eubacterium* coprostanoligenes converts cholesterol into coprostanol in humans, an efficiently excreted form, indicating that a normal microbiome helps maintain cardiometabolic health [44].

Changes in the gut microbiota help to maintain the body's protective barriers and ensure a healthy metabolism. For example, *Bacteroidetes* are involved in the breakdown of carbohydrates and bile acids. *Firmicutes*, especially *Clostridium* and *Lactobacillus*, are known for producing butyrate, an important source of energy for the colon lining and vital for gut health [45-48]. In early childhood, *Bifidobacterium* (a member of the *Actinobacteria* group) stands out for its ability to digest the sugars in human milk. Another important organism, *Akkermansia muciniphila*, contributes to healthy mucus layers and overall metabolic balance [49,50]. Each of these microbes seems to help the digestive system and support the body's natural defenses in its own unique way.

Taken together, current research suggests that the microbiome acts as a bridge between metabolism, the immune system and other body systems. Microbial byproducts like

Table 5 Normal functions of the gut microbiota in several systems

Researcher [ref.]	System affected	Microbial agent/mediator of response	Potential mechanism - main function affected
Fan *et al* [26] Shin *et al* [27] Jiang *et al* [28] Liu *et al* [29]	Immune system	1. *Peptostreptococcus russellii, Lactobacillus spp.*, and *Clostridium sporogenes*	1. Conversion of dietary tryptophan (Trp) into aryl hydrocarbon receptor (AHR) ligands to activate various receptors on immune cells, such as the aryl hydrocarbon receptor (AhR), leading to downstream signaling cascades that regulate immune cell differentiation, cytokine production, and barrier function
		2. Indole-3-propionic acid (IPA)	2. Produced from tryptophan, IPA can affect immune cell function and intestinal barrier permeability
		3. 4-hydroxyphenylpropionic acid (4-OH-PPA)	3. A tyrosine metabolite that has been shown to protect against influenza through a type 1 interferon-dependent mechanism
		4. p-cresol	4. A metabolite of phenylalanine and tyrosine that can contribute to inflammatory processes
He *et al* [30] De Angelis *et al* [31] Schalla *et al* [32] Tennoune *et al* [33] Jiang *et al* [34] Kelly *et al* [35] Vicentini *et al* [36] Schroeder *et al* [37] Wu *et al* [38]	Nervous system (the gut-brain axis)	1. *Bacteroides* (certain species), *Coriobacteriaceae, Veillonellaceae, Prevotella, Bifidobacterium* (certain species), *Lactobacillus* (certain species), *Coprococcus* and *Ruminococcus*	1. Increase ghrelin production, which stimulates appetite and regulates the storage of energy in the form of fat, but also reduces anxiety, stress, and pain
		2. *Bacteroides*	2. Production of molecules homologous to insulin, NPY and melanocyte-stimulation hormone (α-MSH) that induce cross-reactions with immunoglobulins in the circulatory system that act directly against ghrelin, leptin, insulin, PYY and NPY
		3. *Rikenellaceae* and *Clostridiaceae*	3. Production of caseinolytic protease B (ClpB) that mimics satiety
		4. Certain species of *Lactobacillus*	4. Increased production of acetylcholine that works as a neurotransmitter in the enteric nervous system (ENS), also called the "brain within the gut", maintaining peristalsis, and intact gut permeability
		5. *Lactobacillus rhamnosus JB-1*	5. Alterations in expression of GABARs in the brain, which lead to a decrease in anxiety and depression
		6. *E. coli, Hafnia, Bacteroides, Streptococcus, Bifidobacterium, Lactococcus, Lactobacillus, Morganella, Klebsiella, Propionibacterium, Eubacterium, Roseburia* and *Prevotella, Candida* and *Escherichia*	6. Conversion of tryptophan in food to 5-HT, which acts alongside serotonin to affect emotional behavior
		7. *Bifodobacterium, Lactobacillus, Lachnospiraceae, Blautia, Coprococcus, Roseburia* and *Faecalibacterium*	7. Production of SCFAs that interact with GPR43 to stimulate energy expenditure in skeletal muscles and the liver. Restoration of enteric neurons and neuroglia, which inhibit cognitive disorders
		8. Butyrate-producing bacteria	8. Alleviation of depressive behavior, dementia and brain trauma
		9. Increased abundance of *Odoribacter, Oscillibacter,* and decreased abundance of *Alloprevotella, Peptococcus, Oxalobacter, Ruminococcus* (gnavus group), *Collinsella, Slackia, Clostridium sensu stricto 1, Coprococcus 2 & 3, Eubacterium* (eligens group), and *Butyricimonas*	9. Better sleep quality (no insomnia) and decreased sleepiness during the day

(Contd...)

Table 5 *(Continued)*

Researcher [ref.]	System affected	Microbial agent/mediator of response	Potential mechanism - main function affected
Tilg *et al* [39] Liu *et al* [40] Sun *et al* [41]	Liver (the gut-liver axis)	1. *Lactobacillus reuteri* and *Lactobacillus fermentum*	1. Increase of intestine-derived HDL3 neutralized endotoxin in the portal vein, preventing activation of liver macrophages and liver inflammation
		2. *Clostridium, Bifidobacterium,* and *Firmicutes phyla*	2. Production of SCFAs and bacterial metabolites that control hepatic immune responses
		3. *Lactobacillus, Bifidobacterium,* and the *Lachnospiraceae* family	3. Reduction of liver fat, inflammation and injury. Protection against bacterial translocation in cases of liver damage (e.g. cirrhosis)
Bhat *et al* [42] Ettinger *et al* [43] Ren *et al* [44]	Cardiovascular system	1. *Firmicutes phylum* and *Lachnospiraceae* and *Ruminococcaceae* families	1. Increase in SCFA production that helps attenuate control of blood pressure and inflammation
		2. *Lactobacillus* and *Bifidobacterium*	2. Lowering cholesterol and improving endothelial function
		3. *Eubacterium coprostanoligenes*	3. Conversion of cholesterol into coprostanol, which is then excreted by the body
Jandhyala *et al* [45] Shin *et al* [46] Morrison *et al* [47] Rivière *et al* [48] Cuesta *et al* [49] Rodrigues *et al* [50]	Intestines	1. *Bacteroidetes (*e.g., *Bacteroides)*	1. Break down complex carbohydrates, proteins, and bile acids. Produce short-chain fatty acids (SCFAs) like acetate and propionate. Help regulate immune system balance
		2. *Firmicutes (*e.g., *Clostridium, Lactobacillus, Faecalibacterium)*	2. Major producers of butyrate (an SCFA that fuels colonocytes and maintains gut barrier integrity). Support anti-inflammatory immune responses. Assist in vitamin synthesis (e.g. B vitamins, vitamin K)
		3. *Actinobacteria (*e.g., *Bifidobacterium)*	3. Important in infancy (digesting human milk oligosaccharides). Produce acetate and lactate, which other bacteria convert into butyrate. Inhibit pathogens by lowering gut pH
		4. *Proteobacteria (*e.g., *Escherichia coli*, non-pathogenic strains)	4. Contribute to nitrogen metabolism. Overgrowth can indicate dysbiosis (imbalance)
		5. *Verrucomicrobia (*e.g., *Akkermansia muciniphila)*	5. Degrade mucus in the gut lining, stimulating mucus turnover and gut barrier health. Linked to metabolic regulation and reduced inflammation

SCFA, short-chain fatty acid; Trp, tryptophan; IPA, indole-3-propionic acid; 4-OH-PPA, 4-hydroxyphenylpropionic acid; ClpB, caseinolytic protease B; 5-HT, 5-hydroxytryptamine (serotonin); PYY, peptide YY; NPY, neuropeptide Y; ENS, enteric nervous system; GABARs, γ-aminobutyric acid receptors; HDL3, high-density lipoprotein subclass 3; AHR/AhR, aryl hydrocarbon receptor; PUFA, polyunsaturated fatty acid

SCFAs, IPA, and caseinolytic protease B do not just affect the gut: they also shape our immune responses, brain function, liver metabolism and even heart health. Still, much of what we know comes from studies in animals. While research in humans is increasing, the evidence is still limited—often based on small groups of people and short-term studies. Although scientists have mapped some key processes, such as the breakdown of SCFAs and tryptophan, many questions remain about the gut microbiome and its interactions with our bodies. For example, the influence of genetics, medications, sleep patterns and early-life experiences has not been fully explored in most studies.

Collectively, these findings demonstrate that the gut microbiome operates as a dynamic, multisystemic metabolic organ. Its effects extend across immune, neural, hepatic, cardiovascular and intestinal networks. Despite rapid advances, current knowledge remains limited, as a result of heterogeneity in study protocols, small sample sizes, and selective research focused on a narrow subset of microbial metabolites. To better interpret the interactions between microbial pathways and host physiology, larger-scale human studies are needed. Metagenomics, metatranscriptomics, metabolomics, proteomics and immune profiling are all essential components of our deep understanding of the microbiome and its complexity. Such approaches will be essential for distinguishing causal relationships from correlation, identifying temporal signatures of microbial activity, and uncovering currently uncharacterized biochemical pathways. Advancing toward this systems-level understanding will provide the mechanistic resolution needed to translate microbiome research into predictive, personalized, and clinically actionable insights.

Concluding remarks

To conclude, compositional diversity, functional resilience, and adaptability to host and environmental influences are all components of what we call "a healthy gut microbiome". Rather than a fixed taxonomic profile, health corresponds to the preservation of metabolic and immunological functions across different microbial configurations. Future longitudinal and multi-omics studies are essential to refine the boundaries of normality and guide targeted interventions.

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Supplementary material

Supplementary Table 1 Risk of bias – studies' quality assessment

Study [ref.]	Design; n	Major sources of bias/ confounding	Strengths	Limitations	Overall quality
Yu *et al* (2023) [6]	Cross-sectional; n=31	Small n; constipation subgrouping confounds; potential lifestyle/ medication confounding	Clear clinical phenotyping; PMCID available	Small sample, single center, limited correction for diet/meds	Moderate–low
Shalon *et al* (2023) [7]	Physiological sampling study; n=15	Small sample; sampling invasiveness may select special population	Novel sampling (*in situ* intestinal content) and multi-omic approach	Very small n, complex technical workflow, potential sampling bias	Moderate
Pihelgas *et al* (2024) [8]	Interventional (fiber), n=12	Very small n; short-term follow-up; individual baseline effects	Controlled intervention, resilience-focused question	Underpowered for subgroup inference; limited external validity	Moderate–low
Olivares *et al* (2021) [9]	Cross-sectional/ metabolic phenotypes; n=18	Small sample; confounding by unreported meds/diet	Metabolic phenotyping; exclusion of some meds	Small n, limited covariate adjustment	Moderate–low
Xia *et al* (2024) [10]	Case-control in elderly hyperlipidemia; n=10	Very small n; elderly-specific; meds common in elderly	Targeted elderly population; sequencing depth (PE300)	Very small n, selection bias, probable medication confounding	Low
Zhao *et al* (2023) [11]	Large cross-sectional adolescents; n=302	Cross-sectional (no causality); limited adjustment for all lifestyle factors	Large sample, population-level urbanization analysis	Short antibiotic exclusion window; adolescent-specific results	High–moderate
Khachroub *et al* (2023) [12]	Cross-sectional Tunisian; n=19	Small n; limited covariates	First characterization in Tunisian adults	Small sample; generalizability limited	Moderate–low
Zhang *et al* (2022) [13]	Cross-sectional poor sleep vs. controls; n=17	Small n; sleep assessment method (preliminary)	Exploratory link sleep–microbiome	Preliminary sample size; possible multiple testing	Moderate–low
Brooks *et al* (2018) [14]	Large multi-ethnic dataset (s); n≈1375	Heterogeneous datasets, batch effects, varying metadata	Large N, ethnic diversity	Lack of standardized methods; variable sample details	Moderate
Ang *et al* (2021) [15]	Comparative East Asian vs. White; n=46	Modest sample, potential residual confounding	Comparative design controlling for locale	Small n for subgroup analyses	Moderate–low
Yasir *et al* (2015) [16]	Comparative France vs. Saudi Arabia; n=29	Small sample; substantial cultural/diet confounding	Cross-country comparison (novel)	Old study (2015) with small n	Moderate–low
Kulecka *et al* (2020) [17]	Athletes vs. controls; n=81	Athlete lifestyle confounds; training/diet heterogeneity	Larger sample for targeted question; athlete phenotyping	Platform differences (Ion Torrent) vs. others; cross-sectional	Moderate
Takagi *et al* (2022) [18]	Japanese cohort typing; n=283	Cross-sectional; population-specific	Large cohort; broad taxonomic typing	Limited functional measures; generalizability outside Japan	High–moderate