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# Unraveling the Influence of Lifestyle Choices on Microbiota Diversity – **A Comprehensive Review**

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### ABSTRACT

## Introduction

In recent years, there has been a growing interest in the interplay between lifestyle choices, particularly nonpharmacological methods, and their profound impact on gut health. This burgeoning interest is fuelled by a growing body of research highlighting the pivotal role of the gut microbiota in maintaining overall health and wellbeing.

## Aim of the study

The aim of this review is to explore the nexus between an optimal health-related choices and gut microbiota composition, highlighting the latest research findings and insights into how dietary patterns, physical activity, psychological stress and sleep quality influence microbial diversity and functionality.

## Materials and methods

A comprehensive examination of scientific literature available in the PubMed and Google Scholar databases was undertaken. The search strategy incorporated keywords such as "gut", "gut microbiota", "exercise", "diet", "stress" and "circadian rhythm" to identify relevant articles.

## Results

The literature review findings unequivocally reveal an increased diversity in the gut microbial community and a favourable balance between commensal and pathogenic bacteria, as well as overall gut health, including integrity and functionality, attributable to adherence to a healthy lifestyle. These findings highlight the intricate interplay between lifestyle factors and colon microbial composition.

### Conclusions

Although initial findings are encouraging, additional investigation into the potential impact of gut microbiota on health, particularly in the long term, is indispensable. Moreover, the limitations inherent in current research methodologies, such as small sample sizes, variability in study designs, and inconsistencies in findings, underscore the need for robust, well-controlled studies to validate and expand upon existing findings. Robust studies are necessary to develop targeted interventions for optimizing gut health.

Keywords: gut; gut microbiota; exercise; diet; stress; circadian rhythm

#### INTRODUCTION

The intestinal microbiota, also known as gut microbiota, is composed of a diverse array of microorganisms, including bacteria, fungi, viruses, and archaea. Among these, bacteria are the most abundant and extensively studied components. The density of bacterial cells in the colon is estimated to be approximately 10<sup>13</sup> to 10<sup>14</sup> per millilitre, making it one of the most densely populated microbial environments on the planet.<sup>1</sup> At present, the gut microbiota's bacterial component is predominantly composed of four major microbial phyla, accounting for over 90% of its composition: Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria.<sup>2</sup> The majority of beneficial bacteria inhabiting the human gut microbiota belong to gram positive Firmicutes and gram negative Bacteroidetes.<sup>3</sup>

Each species has unique metabolic functions and interactions with the host. The myriad and abundant constituents of the human gut microbiota play indispensable roles in sustaining human health. They facilitate the enzymatic breakdown of dietary substrates, liberating nutrients otherwise inaccessible to the host, aid in host cell differentiation, shield the host against pathogen colonization, and modulate the immune system by eliciting or regulating immune responses.<sup>4</sup>

Furthermore, the composition of the microbiota may be influenced by both endogenous and exogenous factors. The diversity of the gut microbiota can vary widely among individuals and can be influenced by modifiable factors such as: diet<sup>5</sup>, physical activity<sup>1</sup>, psychological stress<sup>6</sup>, sleep<sup>7</sup>, circadian rhythm disorganization<sup>8,9</sup>, medications, and environmental exposures. Maintaining a balanced and diverse gut microbiota is essential for digestive health, immune function, and overall well-being. In this review, we aim to elucidate the contemporary understanding of the health benefits associated with health-promoting behaviours, focusing specifically on their influence on the composition of the gastrointestinal microbiota.

#### **Diet and Gut Microbiota**

In recent years, significant progress has been made in understanding the relationship between diet and the gut microbiome. Recent studies have demonstrated that diet is a main factor in gut microbiome diversity, both over short-term and long-term periods.<sup>10</sup> In this chapter, we will explore recent research elucidating the correlation between dietary factors and microbial diversity.

In a study investigating the correlation between dietary factors and the microbiome across a comprising 1,425 individuals divided into four cohorts: Crohn's disease, ulcerative colitis, irritable bowel syndrome, and the general population. This analysis aimed to unravel the associations between dietary patterns and the composition of the gut microbiome. Unsupervised clustering identified dietary patterns and microbial clusters, with subsequent analysis revealing 38 associations between these patterns and clusters. Additionally, 61 individual foods and nutrients were linked to 61 species and 249 metabolic pathways. Processed and animal-derived foods correlated with higher abundances of Firmicutes, Ruminococcus species of the Blautia genus, and endotoxin synthesis pathways, while plant foods and fish were positively associated with short-chain fatty acid-producing commensals and pathways of nutrient metabolism.<sup>5</sup>

Another study by Naseri et al. aimed to investigate the relationship between dietary intervention and changes in symptoms and diversity in the intestinal microbiota. This clinical trial involved 42 patients diagnosed with irritable bowel syndrome (IBS) based on Rome IV criteria, who underwent a low-FODMAP and gluten-free diet (LF-GFD) intervention for six weeks. Symptoms were assessed using the IBS symptom severity scoring (IBS-SSS), and faecal samples were collected at baseline and post-intervention for analysis using quantitative 16S rRNA PCR assay to evaluate gut microbiota diversity. Not only did the study show a significant reduction in IBS-SSS scores after LF-GFD intervention compared to baseline, but also decent microbial differences before and after the intervention, with a notable increase in Bacteroidetes in analysis of faecal samples. Moreover, the Firmicutes to Bacteroidetes (F/B) ratio significantly decreased.<sup>11</sup>

Chung et al. conducted a study aiming to investigate the effects of supplying two different non-digestible polysaccharides, apple pectin and inulin, as energy sources to three distinct human gut microbial communities in controlled anaerobic fermentors. Analysis revealed specific enrichment of bacterial operational taxonomic units (OTUs). pH variations influenced microbial responses, with shifts observed in dominant bacterial sequences. Pectin notably promoted the enrichment of Eubacterium eligens, a member of the Firmicutes phylum. Additionally, pectin-fed fermentors exhibited greater community diversity compared to inulin-fed fermentors. The findings demonstrate the potential of specific non-digestible dietary carbohydrates to modulate the gut microbiota at the level of individual strains and species, influenced by factors such as pH fluctuations. Identifying bacteria stimulated by prebiotic interventions is essential for enhancing efficacy and safety in promoting human health. <sup>12</sup>

Another study elucidating the impact of pectin on the intestinal microbiota was conducted by Bianchi et al. By the use of Simulator of the Human Intestinal Microbial Ecosystem (SHIME), researchers demonstrated that citrus pectin facilitated the production of butyric acid within the simulated transverse and descending colon. Furthermore, it stimulated the proliferation of genera Lactobacillus, Megamonas, and Lachnospiracea, displaying concomitant anti-inflammatory effects, alongside a reduction in ammonium ion levels.<sup>13</sup> Bonder et al. conducted a study focusing on the effect of a short-term gluten-free diet on gut microbiome. The study examined alterations in the gut microbiomes of 21 healthy volunteers following a gluten-free diet (GFD) for four weeks. Microbiome profiles were determined using 16S rRNA sequencing, and taxonomic and functional composition analyses were conducted. Inter-individual variation in the gut microbiota remained consistent during the short-term GFD intervention. However, notable reductions in Ruminococcus bromii and Roseburia faecis, while showing an increase in Victivallaceae and Clostridiaceae abundances were observed. Several taxon-specific differences were observed during the GFD period, notably a significant reduction in the family Veillonellaceae (class Clostridia). Seven other taxa also exhibited significant changes, with many involved in starch metabolism. Stronger differences were observed in pathway activities, with 21 predicted pathway activity scores showing significant associations with dietary changes. <sup>14</sup>

Haro et al. aimed to investigate changes in the intestinal microbiota in response to the Mediterranean diet (MD) or a low-fat, high-complex carbohydrate diet (LFHCC diet) in an obese population. The study involved 20 obese male participants. One of the primary objectives of the study was to evaluate bacterial composition. Following the Low-Fat High-Complex Carbohydrate (LFHCC) diet, there was an increase in Prevotella and a decrease in Roseburia genera, whereas the Mediterranean diet led to a decrease in Prevotella and an increase in Roseburia and Oscillospira genera. Additionally, the abundance of Parabacteroides distasonis and Faecalibacterium prausnitzii increased after long-term consumption of the MD and LFHCC diets. <sup>15</sup>

#### **Relationship Between Mental Health and Gut Microbiota**

In the study, researchers aimed to assess the impact of a social stressor known as social disruption (SDR) on the bacterial populations in the cecum of mice. The study utilized mice as experimental subjects and exposed them to the social disruption stressor to induce immunoenhancement. Bacterial populations in the cecum were characterized using bacterial tag-encoded FLX amplicon pyrosequencing, a method for profiling microbial communities. The findings of the study revealed significant alterations in the community structure of the microbiota following exposure to the social disruption stressor, particularly evident immediately after stressor exposure. Notably, stressor exposure led to a decrease in the relative abundance of bacteria belonging to the genus Bacteroides, while increasing the relative abundance of bacteria in the genus Clostridium. Additionally, the stressor induced elevations in circulating levels of IL-6 and MCP-1, which were found to be significantly correlated with stressor-induced changes in three bacterial genera, namely Coprococcus, Pseudobutyrivibrio, and Dorea. These results suggest a potential role of the microbiome in mediating stress-induced immunoenhancement.<sup>16</sup>

When it comes to the connection between depression and changes in the intestinal microbiota Huang et al. conducted a study focused on one group of bacteria, namely Firmicutes. The aim of this study was to investigate the alterations and effects of Firmicutes in patients diagnosed with major depressive disorder (MDD), considering the potential role of gut microbiota in influencing mood and behaviour. In this investigation, a total of 54 participants were enrolled, comprising 27 individuals diagnosed with major depressive disorder (MDD). Faecal specimens were obtained from each participant and subjected to 16S rRNA sequencing followed by bioinformatics

analysis to elucidate the composition of the gut microbiota. Firmicutes, a major phylum of gut microbiota known for its role in maintaining intestinal barrier function, was found to be significantly reduced in MDD samples. Additionally, 13 taxonomic biomarkers related to Firmicutes showed significant differences between MDD patients and healthy controls. The study concludes that patients with depression exhibit significant alterations in gut microbiota, particularly a decrease in Firmicutes. It suggests that deficiencies in Firmicutes may contribute to depression through mechanisms such as disruptions in short-chain fatty acids, potentially underpinning the physiological basis of low-level inflammation observed in depression. However, the study acknowledges its limitations, including its cross-sectional design, relatively small sample size, and lack of quantified assessment of diet-related factors despite attempts to control for them.<sup>17</sup>

A systematic comparative analysis of the gut microbiome between patients diagnosed with generalized anxiety disorder (GAD) and healthy controls (HCs) was conducted by Jiang et al., with a focus on assessing microbial richness and diversity, metagenomic composition, and the presence of specific bacterial taxa associated with GAD. Firstly, a cross-sectional study was conducted involving 40 patients with active GAD and 36 healthy controls. Subsequently, a subgroup analysis was performed, comprising 12 antidepressant-naive patients and 22 controls, to validate the initial findings. Finally, a prospective study was carried out, focusing on a subgroup of nine GAD patients analyzed during both the active state of anxiety and remission. Faecal samples were collected from all participants and subjected to metagenomic analysis, including assessments of microbial richness, diversity, and the presence of specific bacterial taxa. The study revealed significant differences in the gut microbiota between patients with GAD and healthy controls. GAD patients exhibited markedly decreased microbial richness and diversity compared to healthy individuals. Furthermore, distinct metagenomic composition was observed in GAD patients, characterized by reduced levels of short-chain fatty acid (SCFA)-producing bacteria associated with a healthy gut microbiome, along with an overgrowth of certain bacterial taxa, including Escherichia-Shigella, Fusobacterium, and Ruminococcus gnavus. Importantly, these microbial dysbiosis patterns persisted even in GAD patients in remission. The findings suggest that targeting the gut microbiome may hold promise as a therapeutic and preventive strategy for GAD.<sup>18</sup>

The subsequent study was conducted by Lin et al. and aimed to investigate the relationship between the gut microbiota composition and the mental state of patients diagnosed with major depressive disorder (MDD), recognizing the potential influence of gut flora on MDD. Faecal samples were collected from MDD patients and faecal microbiota was characterised. The results revealed notable differences in the gut microbiota composition of MDD patients compared to healthy individuals, including a higher abundance of the Firmicutes phylum, lower levels of Bacteroidetes, and increased proportions of Prevotella, Klebsiella, Streptococcus, and Clostridium XI genera. Importantly, the changes in the proportion of Prevotella and Klebsiella were consistent with scores on the Hamilton Depression Rating Scale. Despite the study's conclusions were constrained by the small sample size and potential confounding factors affecting faecal microbiota, further analysis on proportions of Prevotella and Klebsiella in faecal microbial communities for the diagnosis and therapeutic monitoring of MDD should be concerned.<sup>19</sup>

Valles-Colomer et al. investigated the relationship between gut microbial metabolism and mental health, focusing on the bidirectional communication between microbiota, the gut, and the brain. The study surveyed a large cohort from the Flemish Gut Flora Project (n = 1,054) and validated findings in independent datasets (total

n = 1,070). Microbiome features were analyzed for correlations with host quality of life and depression. The study employed metagenomic sequencing of faecal samples to characterize microbial composition and used a modulebased analytical framework to assemble a catalogue of neuroactive potential of gut prokaryotes. The findings revealed consistent associations between specific gut bacteria, such as Faecalibacterium and Coprococcus, with higher quality of life indicators. These bacteria were depleted in individuals with depression, even after adjusting for the effects of antidepressants. Analysis of the microbial synthesis potential identified correlations between the dopamine metabolite 3,4-dihydroxyphenylacetic acid and mental quality of life, as well as a potential role of microbial  $\gamma$ -aminobutyric acid production in depression. The study provides population-scale evidence supporting links between the gut microbiome and mental health, highlighting the importance of considering confounders in such analyses.<sup>20</sup>

The clinical study by Szyszkowicz et al. aimed to investigate the association between microbial changes in cecum contents and social avoidance behaviours, a characteristic feature of depression, as well as proinflammatory variations in socially stressed mice. It sought to explore whether alterations within the gut microbiome and the immune system contribute to the pathogenesis of depression following exposure to chronic social defeat stress. Mice were subjected to daily episodes of social defeat stress or a control condition for ten consecutive days. Three weeks after the last stressor episode, social avoidance behaviours were assessed, and blood, brain, and cecum contents were collected for analysis. The study utilized sequencing techniques to characterize microbial communities at various taxonomic ranks. Mice exhibiting the most pronounced social avoidance behaviours, indicative of susceptibility to chronic social defeat stress, showed significant alterations in specific sets of bacteria at the phylum and genus taxonomic levels in their cecum contents. Although plasma and brain cytokine levels did not show significant changes, mRNA expression of interleukin (IL)-1 $\beta$  and IL-6 in the prefrontal cortex correlated with variations in the abundance of Flavobacterium spp. and Turicibacter spp., respectively. These findings suggest a potential link between certain clusters of bacterial communities in cecum contents and vulnerability to social deficits resulting from prolonged exposure to social stressors, although further research is needed to establish causality.<sup>21</sup>

### **Relationship Between Sleep and Gut Microbiota**

Among various factors influencing the distribution, composition, and diversity of intestinal flora, sleep remains one of the less comprehensively understood factors. As far as we know, sleep deprivation exacerbates the migration of viable bacteria from the intestinal tract, presenting an additional mechanism through which interactions between sleep and microbes influence neurobiology. <sup>22</sup>

In a study Everson aimed to investigate the underlying cause of the hypercatabolic state, secondary malnutrition symptoms, and mortality observed in rats subjected to prolonged sleep deprivation. Rats underwent extended periods of sleep deprivation, and various physiological parameters including intermediary metabolism, clinical chemistry, haematological indexes, and postmortem examinations were evaluated. Blood cultures were also conducted to detect invasion by opportunistic microbes. Prolonged sleep deprivation in rats led to a cascade of events culminating in mortality. Despite the absence of gross detectable disturbances in intermediary

metabolism or structural damage, blood cultures revealed invasion by opportunistic microbes, predominantly originating from the gut, including species such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus agalactiae, and Corynebacterium jejeikum, suggesting a breakdown of host defence against indigenous and pathogenic microorganisms as the underlying cause of the observed life-threatening condition. Sleep-deprived rats exhibited significantly higher populations of live aerobic bacteria in the ileum and cecum compared to control groups. Additionally, there was an increase in facultative anaerobic bacteria in the ileum of sleep-deprived rats. The study also observed elevated levels of Enterobacter and Klebsiella pneumoniae in the intestinal homogenates of sleep-deprived rats, indicating microbial dysbiosis induced by sleep deprivation.<sup>21,24</sup> While previous studies have indicated a potential correlation between sleep deprivation, intestinal bacterial translocation, and immune system dysfunction, the validity of the "disc over water" model has faced criticism. Nonetheless, further investigation is essential to validate and elucidate this hypothesis.<sup>22</sup>

The clinical trial on the impact of environmental disruption of normal sleep patterns, resembling shift work or chronic jet lag, on the diurnal rhythmicity and composition of gut microbiota in mice was conducted by Thaiss et al. Mice were subjected to a jet lag model involving an 8-hour time shift every 3 days to simulate frequent changes in light-dark cycles. Diurnal variations in microbiota composition were analyzed through taxonomic analysis of faecal samples collected every 6 hours during the jet lag induction period. Rhythmicity of microbiota composition was assessed using JTK\_cycle analysis. Additionally, the impact of prolonged jet lag on microbiota composition was evaluated by monitoring mice for up to 16 weeks of continuous time shifting. Environmental disruption of normal sleep patterns, as induced by the jet lag model, led to loss of diurnal rhythmicity in both host behaviour, and microbiota composition in mice. Similar to genetic disruption of the circadian clock, jet lag resulted in dysbiosis characterized by altered microbiota composition. Prolonged exposure to jet lag exacerbated dysbiosis, particularly affecting taxonomic units that exhibit diurnal oscillations under normal conditions. These findings suggest that both genetic and environmental disturbances of the mammalian dark-light cycle disrupt feeding rhythms and microbiota rhythmicity, leading to significant alterations in microbiota composition.<sup>25</sup>

#### **Relationship Between Physical activity and Gut Microbiota**

ALLEN et al. conducted an in vivo study with the objective of investigation the effects of endurance exercise training on the composition, functional capacity, and metabolic output of the gut microbiota in both lean and obese individuals. Thirty-two participants, comprising 18 lean (9 female) and 14 obese (11 female) individuals, underwent six weeks of supervised endurance-based exercise training, progressively increasing from 30 to 60 minutes per day at moderate (60% of heart rate reserve [HRR]) to vigorous intensity (75% HRR), followed by a six-week sedentary washout period. Faecal samples were collected before and after the exercise intervention, as well as after the washout period, with three-day dietary controls preceding each collection. The composition, functional capacity, and metabolic output of the gut microbiota were analyzed using beta diversity analysis, faecal concentration measurements of short-chain fatty acids (SCFAs), and assessment of bacterial genes and taxa related to SCFA production. Exercise-induced alterations in the metabolic output of the microbiota were consistent with changes in bacterial genes and taxa involved in SCFA production. Additionally, the study demonstrated that the effects of exercise on the gut microbiota composition and function were influenced by the participants' obesity

status. In lean individuals, exercise increased faecal concentrations of SCFAs, whereas no significant changes were observed in obese participants. Importantly, these exercise-induced changes in the gut microbiota were largely reversed once exercise training ceased, suggesting the dynamic nature of exercise-induced modifications on the gut microbiota. <sup>26</sup>

The clinical study on mice was brought by Lambert et al. The study aimed to investigate the influence of exercise on the cecal microbiota composition in type 2 diabetic (db/db) and control (db/+) mice, with a focus on identifying potential differences between sedentary and exercise conditions. Male type 2 diabetic db/db mice and control db/+ littermates were randomized into sedentary (Sed) or exercise (Ex) groups for a period of 6 weeks. Exercise intervention involved low-intensity treadmill running sessions for 5 days per week, tailored to the physical capacity of the mice. Subsequently, cecal matter was collected and analyzed for total bacterial DNA using quantitative PCR (qPCR) with SYBR Green and group-specific primers. The study revealed significant alterations in the cecal microbiota composition in response to exercise, with notable differences observed between sedentary and exercise groups. Exercise was associated with increased abundance of select Firmicutes species and decreased levels of Bacteroides/Prevotella spp. in both normal and diabetic mice. Additionally, the study highlighted an interaction effect between exercise and diabetes status on the abundance of Bifidobacterium spp. Exercise-induced changes in the gut microbiota warrant further investigation to elucidate the underlying mechanisms and potential implications for metabolic health. <sup>27</sup>

A clinical study involving human subjects aimed to assess the influence of physical exercise on the gut microbiota composition. Female participants engaged in regular physical exercise and sedentary individuals were recruited for the study. Gut microbiota composition was analyzed using high-throughput sequencing of the 16S rRNA gene. Additionally, quantitative PCR (qPCR) analysis was conducted to quantify the abundance of particular bacterial species known for their health-promoting effects. Correlation analysis was performed to evaluate the relationship between exercise-related parameters, such as body fat percentage, muscular mass, and physical activity levels, and bacterial populations. The study findings demonstrated notable differences in gut microbiota composition between active and sedentary women. Active individuals displayed a higher abundance of beneficial bacterial species, including Faecalibacterium prausnitzii, Roseburia hominis, and Akkermansia muciniphila. Moreover, several bacterial populations showed correlations with body fat percentage, muscular mass, and physical activity levels. These results imply a potential interdependence between sedentary behaviour parameters and specific bacterial genera, underscoring the potential influence of both exercise engagement and the disruption of sedentary habits on gut microbiota composition.<sup>28</sup>

The subsequent study was conducted by Petersen et al. and focused on characterization of the gut microbiomes and potential differences between professional and amateur cyclists. A total of thirty-three cyclists were recruited for the study, and their gut microbiomes were analyzed using mWGS and RNA-Seq sequencing. Taxonomic clustering was performed based on mWGS data to identify prevalent microbial genera. Correlation analyses were conducted to assess associations between taxonomic clusters, cyclist status (professional vs. amateur), and exercise duration. Metatranscriptomic analysis was utilized to examine gene expression patterns within the gut microbiomes and identify active metabolic pathways. The study identified three taxonomic clusters in the gut microbiomes of cyclists, characterized by high abundance of Prevotella, high abundance of Bacteroides, or a mix of various genera including Bacteroides, Prevotella, Eubacterium, Ruminococcus, and Akkermansia.

While no significant correlation was found between taxonomic cluster and cyclist status, high Prevotella abundance was associated with increased exercise duration. Metatranscriptomic analysis revealed differences in gene expression patterns, particularly an upregulation of Methanobrevibacter smithii transcripts in professional cyclists. This archaeon showed increased expression of methane production genes, along with upregulation of energy and carbohydrate metabolism pathways, suggesting potential metabolic benefits in the gut microbiome of professional cyclists.<sup>29</sup>

Clarke et al. investigated the impact of extreme exercise, commonly observed in professional athletes, on the diversity of gut microbiota. The study recruited professional athletes from an international rugby union squad along with a control group matched for physical size, age, and gender. Compositional analysis of the gut microbiota was conducted using 16S rRNA amplicon sequencing. Each participant completed a detailed food frequency questionnaire to assess dietary habits. Plasma creatine kinase levels, a marker of extreme exercise, as well as inflammatory and metabolic markers, were measured to characterize the participants' physiological status. The study found significant differences between athletes and controls in plasma creatine kinase levels, inflammatory markers, and metabolic markers, confirming the impact of extreme exercise on physiological parameters. Importantly, athletes exhibited a higher diversity of gut microorganisms, with representation from 22 distinct phyla. Compared to the high BMI individuals, elite athletes displayed significantly higher proportions of 48 taxa. Among the top six flux changes in relative abundance, prominent shifts were observed in Firmicutes, Ruminococcaceae, S24-7, Succinivibrionaceae, RC9 gut group, and Succinivibrio groups. Notably, elite athletes exhibited significantly higher proportions of Akkermansiaceae (family) and Akkermansia (genus) compared to high BMI controls. Only one taxon, Bacteroidetes, was notably less abundant in elite athletes compared to high BMI individuals. Compared to the low BMI individuals, elite athletes demonstrated significantly higher proportions of 40 taxa and lower proportions of three taxa (Lactobacillaceae, Bacteroides, and Lactobacillus). The top six flux changes in relative abundance were observed among the Prevotellaceae, Erysipelotrichaceae, S24-7, Succinivibrionaceae, Prevotella, and Succinivibrio groups. This increased microbial diversity positively correlated with protein consumption and plasma creatine kinase levels. These findings suggest a beneficial impact of exercise on gut microbiota diversity. However, the study also highlights the complexity of this relationship, indicating that it is influenced by accompanying dietary patterns, especially those associated with extreme exercise regimens.<sup>30</sup>

Cataldi et al. aimed to systematically review existing scientific evidence on the bidirectional relationship between physical activity/exercise (PA/PE) and the human gut microbiome (GM), with a specific focus on understanding how different types and variables of PA/PE, as well as age-related effects, impact the GM in both healthy and unhealthy individuals. A systematic search was conducted across four databases (Web of Science, Medline (PubMed), Google Scholar, and Cochrane Library) using predefined search terms. Information was extracted following the PICOS format (populations, exposure, intervention, comparison, outcomes). Quality assessment was performed using the Oxford Quality Scoring System Scale, the Risk of Bias in Non-Randomized Studies of Interventions (ROBINS-I) tool, and the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies. Data extracted included author, publication year, study design, participant characteristics, type of PA/PE, intervention details, measurement tools, and main outcomes. The synthesis of findings from the reviewed studies indicates several key associations between PA/PE and the human GM. Specifically, aerobic exercise is associated with increased GM diversity, whereas resistance training does not show the same effect. The abundance of the Prevotella genus appears to correlate with training duration. Exercising according to the minimum dose recommended by the World Health Organization does not significantly alter GM richness and diversity. However, intense and prolonged PE may lead to a higher abundance of pro-inflammatory bacteria. Additionally, PA does not significantly impact GM alpha/beta diversity in elderly individuals (aged 60+ years). The heterogeneity of training parameters, diet control, and sequencing methods are identified as main confounders in the reviewed studies. <sup>31</sup>

#### CONCLUSIONS

The article emphasizes the complex and multifaceted nature of the gut microbiota and provides evidence supporting the connection between the composition of intestinal microbiota and behaviours that promote health. Understanding the interplay between various lifestyle factors and the gut microbiota could pave the way for targeted interventions aimed at optimizing gut health and preventing associated health conditions. It is imperative to highlight that while the findings present promising insights, additional research is warranted, particularly concerning the direct influence of health-promoting behaviours, beyond diet, on the cultivation of specific bacterial populations. The constraints linked to individual health behaviours imply the need for further investigation to comprehensively elucidate the mechanisms governing gut bacterial diversity and their direct association with health outcomes.

#### **Author's contribution**

Conceptualization, Tomasz Seredyński , Wojciech Mądry and Aleksandra Mazurkiewicz; Methodology, Magdalena Kołodziej; Software, Joanna Męczyńska; Check, Joanna Męczyńska, Nazarii Saiuk and Michał Andrzej Kozicz; Formal Analysis, Adriana Wojciechowska and Justyna Marcicka; Investigation, Weronika Salasa and Justyna Marcicka; Resources, Aleksandra Mazurkiewicz; Data Curation, Wojciech Mądry; Writing -Rough Preparation, Tomasz Seredyński; Writing - Review and Editing, Michał Andrzej Kozicz and Weronika Salasa; Visualization, Tomasz Seredyński; Supervision, Magdalena Kołodziej; Project Administration, Joanna Męczyńska; Receiving Funding, Aleksandra Mazurkiewicz.

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### References

- 1. Gubert C, Kong G, Renoir T, Hannan AJ. Exercise, diet and stress as modulators of gut microbiota: Implications for neurodegenerative diseases. Neurobiol Dis. 2020;134:104621. doi:10.1016/j.nbd.2019.104621
- 2. Bibbò S, Ianiro G, Giorgio V, et al. The role of diet on gut microbiota composition. *Eur Rev Med Pharmacol Sci.* 2016;20(22):4742-4749. http://www.ncbi.nlm.nih.gov/pubmed/27906427
- 3. Singh RK, Chang HW, Yan D, et al. Influence of diet on the gut microbiome and implications for human health. *J Transl Med*. 2017;15(1):73. doi:10.1186/s12967-017-1175-y
- 4. Milani C, Duranti S, Bottacini F, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Published online 2017. doi:10.1128/MMBR
- 5. Bolte LA, Vich Vila A, Imhann F, et al. Long-term dietary patterns are associated with pro-inflammatory and anti-inflammatory features of the gut microbiome. *Gut*. 2021;70(7):1287-1298. doi:10.1136/gutjnl-2020-322670
- 6. Wong ML, Inserra A, Lewis MD, et al. Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition. *Mol Psychiatry*. 2016;21(6):797-805. doi:10.1038/mp.2016.46
- 7. Matenchuk BA, Mandhane PJ, Kozyrskyj AL. Sleep, circadian rhythm, and gut microbiota. *Sleep Med Rev.* 2020;53:101340. doi:10.1016/j.smrv.2020.101340
- 8. Voigt RM, Forsyth CB, Green SJ, et al. Circadian Disorganization Alters Intestinal Microbiota. *PLoS One*. 2014;9(5):e97500. doi:10.1371/journal.pone.0097500
- 9. Voigt RM, Forsyth CB, Green SJ, Engen PA, Keshavarzian A. Circadian Rhythm and the Gut Microbiome. In: ; 2016:193-205. doi:10.1016/bs.irn.2016.07.002
- 10. Beam A, Clinger E, Hao L. Effect of Diet and Dietary Components on the Composition of the Gut Microbiota. *Nutrients*. 2021;13(8):2795. doi:10.3390/nu13082795
- 11. Naseri K, Dabiri H, Rostami-Nejad M, et al. Influence of low FODMAP-gluten free diet on gut microbiota alterations and symptom severity in Iranian patients with irritable bowel syndrome. *BMC Gastroenterol*. 2021;21(1):292. doi:10.1186/s12876-021-01868-5
- 12. Chung WSF, Walker AW, Louis P, et al. Modulation of the human gut microbiota by dietary fibres occurs at the species level. *BMC Biol*. 2016;14(1):3. doi:10.1186/s12915-015-0224-3
- 13. Bianchi F, Larsen N, de Mello Tieghi T, et al. Modulation of gut microbiota from obese individuals by in vitro fermentation of citrus pectin in combination with Bifidobacterium longum BB-46. *Appl Microbiol Biotechnol*. 2018;102(20):8827-8840. doi:10.1007/s00253-018-9234-8
- 14. Bonder MJ, Tigchelaar EF, Cai X, et al. The influence of a short-term gluten-free diet on the human gut microbiome. *Genome Med*. 2016;8(1):45. doi:10.1186/s13073-016-0295-y
- 15. Haro C, Montes-Borrego M, Rangel-Zúñiga OA, et al. Two Healthy Diets Modulate Gut Microbial Community Improving Insulin Sensitivity in a Human Obese Population. *J Clin Endocrinol Metab.* 2016;101(1):233-242. doi:10.1210/jc.2015-3351
- 16. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain Behav Immun.* 2011;25(3):397-407. doi:10.1016/j.bbi.2010.10.023

- 17. Huang Y, Shi X, Li Z, et al. Possible association of Firmicutes in the gut microbiota of patients with major depressive disorder. *Neuropsychiatr Dis Treat*. 2018;Volume 14:3329-3337. doi:10.2147/NDT.S188340
- 18. Jiang H yin, Zhang X, Yu Z he, et al. Altered gut microbiota profile in patients with generalized anxiety disorder. *J Psychiatr Res.* 2018;104:130-136. doi:10.1016/j.jpsychires.2018.07.007
- 19. Lin P, Ding B, Feng C, et al. Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. *J Affect Disord*. 2017;207:300-304. doi:10.1016/j.jad.2016.09.051
- 20. Valles-Colomer M, Falony G, Darzi Y, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat Microbiol*. 2019;4(4):623-632. doi:10.1038/s41564-018-0337-x
- 21. Szyszkowicz JK, Wong A, Anisman H, Merali Z, Audet MC. Implications of the gut microbiota in vulnerability to the social avoidance effects of chronic social defeat in male mice. *Brain Behav Immun.* 2017;66:45-55. doi:10.1016/j.bbi.2017.06.009
- 22. Krueger JM, Opp MR. Sleep and Microbes. In: ; 2016:207-225. doi:10.1016/bs.irn.2016.07.003
- 23. Everson CA. Sustained sleep deprivation impairs host defense. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 1993;265(5):R1148-R1154. doi:10.1152/ajpregu.1993.265.5.R1148
- 24. Everson CA, Toth LA. Systemic bacterial invasion induced by sleep deprivation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.* 2000;278(4):R905-R916. doi:10.1152/ajpregu.2000.278.4.R905
- 25. Thaiss CA, Zeevi D, Levy M, et al. Transkingdom Control of Microbiota Diurnal Oscillations Promotes Metabolic Homeostasis. *Cell*. 2014;159(3):514-529. doi:10.1016/j.cell.2014.09.048
- 26. ALLEN JM, MAILING LJ, NIEMIRO GM, et al. Exercise Alters Gut Microbiota Composition and Function in Lean and Obese Humans. *Med Sci Sports Exerc*. 2018;50(4):747-757. doi:10.1249/MSS.00000000001495
- 27. Lambert JE, Myslicki JP, Bomhof MR, Belke DD, Shearer J, Reimer RA. Exercise training modifies gut microbiota in normal and diabetic mice. *Applied Physiology*, *Nutrition, and Metabolism*. 2015;40(7):749-752. doi:10.1139/apnm-2014-0452
- 28. Bressa C, Bailén-Andrino M, Pérez-Santiago J, et al. Differences in gut microbiota profile between women with active lifestyle and sedentary women. *PLoS One*. 2017;12(2):e0171352. doi:10.1371/journal.pone.0171352
- 29. Petersen LM, Bautista EJ, Nguyen H, et al. Community characteristics of the gut microbiomes of competitive cyclists. *Microbiome*. 2017;5(1):98. doi:10.1186/s40168-017-0320-4
- 30. Clarke SF, Murphy EF, O'Sullivan O, et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut.* 2014;63(12):1913-1920. doi:10.1136/gutjnl-2013-306541
- 31. Cataldi S, Bonavolontà V, Poli L, et al. The Relationship between Physical Activity, Physical Exercise, and Human Gut Microbiota in Healthy and Unhealthy Subjects: A Systematic Review. *Biology (Basel)*. 2022;11(3):479. doi:10.3390/biology11030479