

# Systematic Review on the Gastrointestinal Microbiome in the Diagnosis and Treatment of Anorexia Nervosa

Emilie Mousson<sup>a</sup>, Fieke Van den Broeck<sup>b</sup>, Katrien Van der Vaerent<sup>a,b</sup>, Jaan Toelen<sup>c,d,e</sup>

<sup>a</sup> University Colleges Leuven Limburg, Belgium

<sup>b</sup> Department of Nutrition and Dietetics, University Hospitals Leuven, Belgium

<sup>c</sup> Department of Paediatrics, University Hospitals Leuven, Belgium

<sup>d</sup> Department of Development and Regeneration, KU Leuven, Belgium

<sup>e</sup> KU Leuven Child and Youth Institute, KU Leuven, Leuven, Belgium

emilie.mousson@skynet.be

## Keywords

Anorexia nervosa; Gut microbiome

## Abstract

### Introduction

Anorexia nervosa is a common eating disorder, with a lifetime prevalence of 0.8-6.3% in women and 0.1-0.3% in men. Recently, increasing literature has emerged on insights regarding the gastrointestinal microbiome in these patients. Scientific studies suggest a potential link between the composition of this microbiome and psychological traits such as body dissatisfaction, as well as the influence of this composition on weight evolution.

### Objectives

This systematic literature review aims to present the available information on the gastrointestinal microbiome in patients with anorexia nervosa. Based on these findings, future research may focus on potentially externally modulating the gastrointestinal microbiome to enhance treatment and/or weight gain.

### Methodology

The articles included in this literature review were obtained by consulting scientific databases (PubMed, Embase, CINAHL, and Google Scholar). Out of the 1.189 resulting articles, a selection procedure led to the inclusion of fourteen articles in this review.

### Results

This literature review shows that the gastrointestinal microbiome of patients with anorexia nervosa at the time of diagnosis exhibits reduced richness (Chao1 index, alpha diversity) and an altered composition (beta diversity) compared to healthy individuals. After treatment, alpha-diversity seems to be increased. The microbiome composition evolves but does not normalize after treatment. The altered microbiome composition appears to have significant implications regarding weight gain.

### Conclusions

This literature review highlights a clear deviation in both the composition and richness of the gastrointestinal microbiome in patients with anorexia nervosa compared to healthy individuals. External modulation of the microbiome offers a potential complementary therapeutic approach for these patients.

## Introduction

Anorexia nervosa (AN) is a common eating disorder, affecting 0.8-6.3% of women and 0.1-0.3% of men worldwide at some point in their lives (1). In Belgium, data from 2022-2023 indicate that 13% of the population aged 10 to 64 years exhibits disordered eating behaviour, with the highest prevalence (18%) among adolescent girls (2). Despite growing knowledge about anorexia nervosa, it remains one of the most lethal psychiatric disorders, with an average mortality rate of 5% within the first four years after diagnosis (3). AN is characterized by an intense fear of gaining weight, a severely distorted body image and low self-esteem. These factors result in insufficient energy intake, leading to significantly low body weight relative to age, sex, growth trajectory and physical health. Two subtypes of anorexia nervosa can be distinguished: the restrictive type and the binge-eating/purging type. The

restrictive type is defined by weight loss resulting from strict dieting, fasting and/or excessive physical activity. In contrast, the binge-eating/purging type involves recurrent episodes of binge eating and/or purging behaviours (e.g., vomiting or the use of laxatives) (4). Scientific studies have reported associations between the abundance of specific microorganisms and psychological traits related to anorexia nervosa, such as body dissatisfaction and a drive for thinness (4, 5). These findings offer new perspectives on the potential interaction between disordered eating behaviour and the gastrointestinal microbiome in patients with anorexia nervosa. The gastrointestinal microbiome refers to the community of microorganisms residing in the gut, which plays a crucial role in digestion, immunity and other psychological processes (6). The interaction between gut microorganisms and brain function can be explained by the gut-brain axis. This refers to a bidirectional communication system between

the gut and the brain. The gastrointestinal microbiome can influence brain function through various mechanisms, including the production of short-chain fatty acids and the synthesis of neurotransmitters. Conversely, signals from the brain can also influence gastrointestinal function and the composition of gut microorganisms (7). The gastrointestinal microbiome can be evaluated using various parameters. For the purposes of this literature review, alpha and beta diversity are key concepts. Alpha diversity describes the microbial composition within a single individual (8). Higher alpha diversity indicates a greater number of species, a more balanced distribution and/or greater phylogenetic diversity (i.e. how evolutionarily distinct the species are from one another) (8). This can be assessed using measures such as the Shannon Index (to evaluate the distribution) and the Chao1 Index (to estimate species richness) (9). Research has shown that a less balanced distribution of microorganisms is associated with more severe depressive symptoms (10). This suggests that an increase in alpha diversity among patients could be associated with clinical improvement. Conversely, beta diversity compares the microbiome composition between individuals. It reflects how distinct the gut microbial communities are between different people (8). A reduction in beta diversity between a healthy population and a patient group may indicate a shift in the microbial composition of patients toward the profile of healthy people. The aim of this literature review is to synthesize the current knowledge on the gastrointestinal microbiome in patients with anorexia nervosa. By critically analysing and comparing the existing literature, valuable insight may be identified for future research and potential therapeutic applications.

## Materials and Methods

### Protocol

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (11). The primary objective of this review was to synthesize findings from studies investigating the gastrointestinal microbiome in patients with anorexia nervosa. In the included studies, the gastrointestinal microbiome was assessed through stool sample analyses from both patient (AN) and healthy control populations (HC).

### Stool analyses

Most of the included studies employed Polymerase Chain Reaction (PCR) to analyse stool samples, specifically targeting the 16S or 23S rRNA gene. This technique uses the enzyme DNA polymerase, which synthesizes a complementary DNA strand based on an existing DNA template. Once amplification is complete, the DNA can be further analysed to identify the specific microorganisms present in the microbiome of the examined samples (12). In addition, Fouladi et

al. utilized next-generation sequencing (NGS), specifically shotgun-metagenomic-sequencing (13). This analytical method involves extracting DNA from all cells within a community, fragmenting it into smaller pieces, and then individually sequencing and analysing these fragments (14).

### Search strategy and study selection

A literature search was conducted to identify relevant articles for this review. For this purpose, database-specific search strings were developed, and the databases were consulted on October 18, 2024. The databases used for this review were PubMed, Embase, CINAHL and Google Scholar. The formulation of search strings was guided by Emtree (Embase) and MeSH (PubMed). Based on the synonym lists and hierarchical term structures provided by these tools, relevant alternative terms were identified and incorporated. These search strings were subsequently adapted to the syntax requirements of each database to yield the most relevant results (see supplementary material).

### Inclusion and Exclusion criteria

The inclusion criteria for this review were all studies that were performed in humans with anorexia nervosa and studied the gastrointestinal microbiome using stool samples. Studies were excluded based on the following criteria: (a) study design: review articles or study protocols without results; (b) population: animal or in vitro models, or studies with a sample size smaller than five participants; (c) scope: insufficient specificity regarding the research topic; (d) accessibility; full-text not available.

### Data extraction

Data extraction was performed manually by the researchers. The following information was collected: population characteristics (e.g., mean age, mean BMI, gender, sample size), method of faecal analysis, diagnostic criteria, reported findings related to the gastrointestinal microbiome, and relevant interpretations or recommendations.

### Primary outcome

The primary outcome of this systematic review was to synthesize reported findings regarding the gastrointestinal microbiome in patients with anorexia nervosa, both at the time of diagnosis and after treatment. For this purpose, the microbiome of patients at diagnosis was compared with that of healthy individuals. Additionally, changes in the microbiome during treatment were assessed by comparing the gastrointestinal microbiome of patients after treatment with that at the time of diagnosis.

TABLE 1: Characteristics of the included studies.

| Author                           | Year | Population<br>(AN0/AN1/HC) <sup>1</sup> | BMI (kg/m <sup>2</sup> ) |                        |                        |  | Age (years)         |                        |                        | Duration disease             | Duration treatment         |
|----------------------------------|------|-----------------------------------------|--------------------------|------------------------|------------------------|--|---------------------|------------------------|------------------------|------------------------------|----------------------------|
|                                  |      |                                         | AN <sup>0</sup>          | AN <sup>1</sup>        | HC                     |  | AN <sup>0</sup>     | AN <sup>1</sup>        | HC                     |                              |                            |
| Andreani et al. (27)             | 2024 | 57/50/34                                | 15.99<br>(12.50;18.77)   | 18.88<br>(16.85;20.96) | 20.94<br>(17.65;26.07) |  | 16.25<br>(12;20.35) | 16.52<br>(12.23;19.01) | 16.56<br>(14.11;18.46) | 15.25<br>(1.63;71.97) months | //                         |
| Armougom et al. (16)             | 2009 | 9/-/20                                  | 12.73 ± 1.602            | -                      | 20.68 ± 2.014          |  | 19 - 36             | -                      | 13 - 68                | //                           | //                         |
| Borgo et al. (26)                | 2017 | 15/-/15                                 | 13.9 ± 2.1               | -                      | 22.1 ± 2.6             |  | -                   | -                      | -                      | //                           | //                         |
| Fan et al. (5)                   | 2023 | 77/-/70                                 | 15.6 ± 2.5               | -                      | 21.8 ± 1.9             |  | 23.5 ± 7.0          | -                      | 23.2 ± 4.4             | 5.5 years                    | //                         |
| Fouladi et al. (13) <sup>2</sup> | 2022 | 93/78/98                                | (38) 15.74 ± 2.11        | (33) 18.28 ± 1.05      | (69) 21.98 ± 2.13      |  | (38) 23.2 ± 6.87    | (33) 22.6 ± 6.90       | (69) 23.6 ± 5.28       | //                           | (33) 32.37<br>± 26.21 days |
|                                  |      | UNC: 38/33/69<br>Denver: 55/45/29       | (55) 13.78 ± 1.74        | (45) 15.41 ± 1.10      | (29) 22.56 ± 1.60      |  | (55) 27.7 ± 7.41    | (45) 27.4 ± 6.68       | (29) 31.1 ± 5.64       |                              | (45) 17.66<br>± 9.56 days  |
| Kleiman et al. (15)              | 2015 | 16/10/12                                | 16.2 ± 1.5               | 17.4 ± 0.9             | 21.5 ± 1.9             |  | 28.0 ± 11.7         | //                     | 29.8 ± 11.6            | //                           | //                         |
| Mack et al. (24)                 | 2016 | 55/44/55                                | 15.3 ± 1.4               | 17.7 ± 1.4             | 21.6 ± 2.0             |  | 23.8 ± 6.8          | //                     | 23.7 ± 6.7             | //                           | 14.0 ± 6.8 weeks           |
| Monteleone et al. (21)           | 2021 | 17/-/20                                 | 15.0 ± 1.8               | -                      | 20.3 ± 1.4             |  | 20.5 ± 3.1          | -                      | 23.0 ± 3.3             | //                           | //                         |
| Monteleone et al. (22)           | 2020 | 21/16/20                                | 14.6 ± 1.3               | 20.5 ± 0.7             | 20.3 ± 1.4             |  | 21.7 ± 4.2          | 22.3 ± 4.4             | 23.0 ± 3.3             | //                           | 20 weeks                   |
| Morita et al. (17)               | 2015 | 14/-/21                                 | 12.7 ± 1.5               | -                      | 20.5 ± 2.1             |  | 28.1 ± 10.7         | -                      | 31.5 ± 7.4             | //                           | //                         |
| Mörkl et al. (19)                | 2017 | 18/-/26                                 | 15.29 ± 1.28             | -                      | 21.89 ± 1.73           |  | 22.44 ± 3.20        | -                      | 24.93 ± 3.75           | 3.14 ± 3.51 years            | //                         |
| Prochazkova et al. (28)          | 2021 | 52/52/67                                | 14.4 (13.4;15.9)         | 17.1 (15.5;18.1)       | 21.9 (19.9;23.7)       |  | 23 (19;27)          | //                     | 24 (22;28.5)           | 60 (36;126) months           | 51 (38.5;64) days          |
| Schulz et al. (25)               | 2020 | 19/19/20                                | 15.76 ± 2.03             | 18.8 ± 0.87            | 20.31 ± 2.35           |  | 15.77 ± 1.94        | 16.16 ± 1.99           | 16.35 ± 1.11           | 17.16 ± 16.12 months         | 4.05 ± 1.39 months         |
| Yuan et al. (23)                 | 2022 | 30/-/30                                 | 14.92 ± 2.54             | -                      | 20.89 ± 2.14           |  | 16 (13;22)          | -                      | 18 (13;24)             | //                           | 46 (17;58) days            |

<sup>1</sup> AN0 : patients with anorexia nervosa at time of diagnosis; AN1: patients with anorexia nervosa

after treatment; HC: healthy controls.

<sup>2</sup> Fouladi et al.s' study was conducted at two centres. the UNC Center of Excellence for Eating Disorders and the Denver ACUTE.

- : not investigated

//: not specified

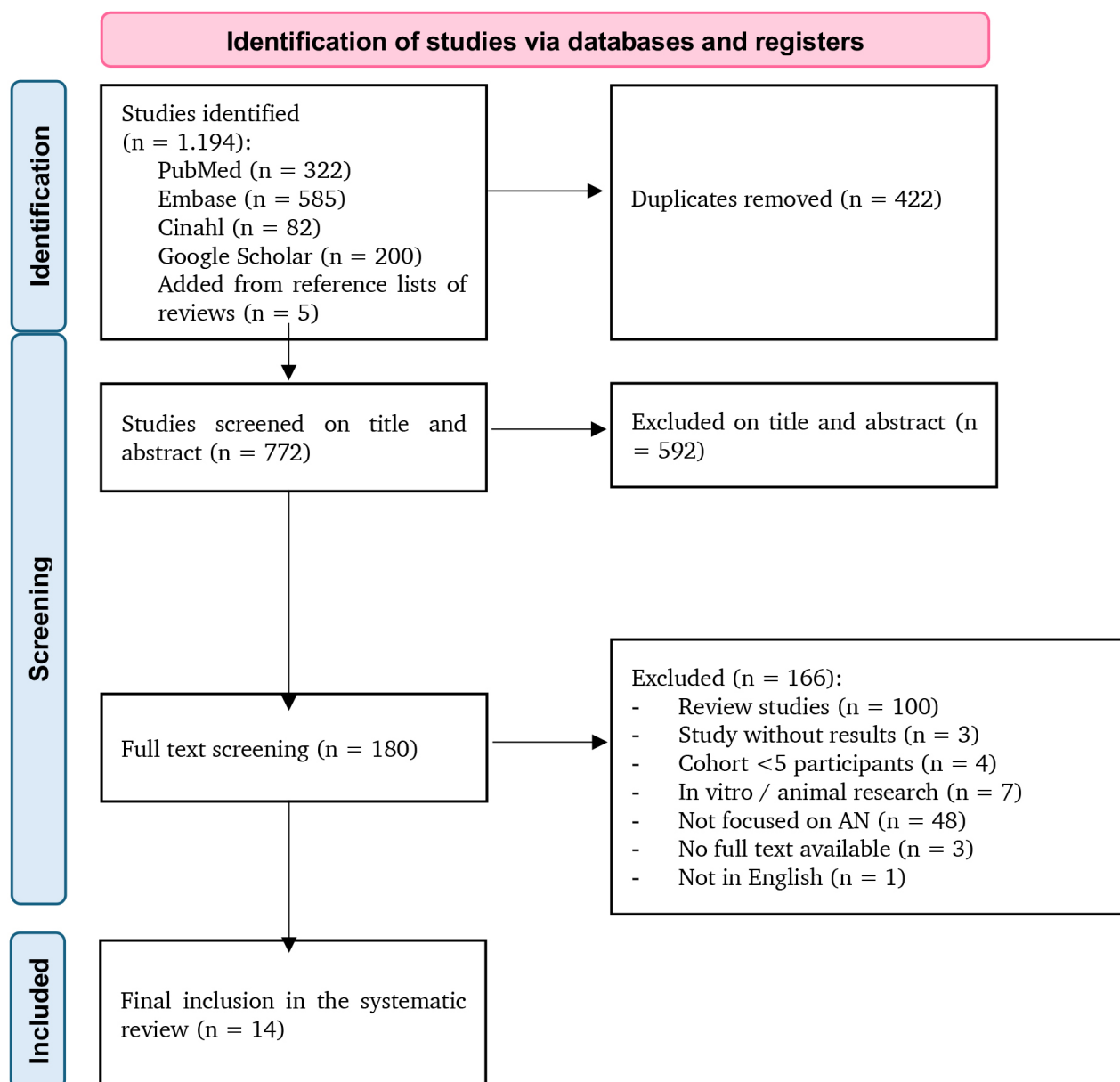


FIGURE 1: PRISMA flowchart.

## Results

### Study selection

The initial search across PubMed, Embase, CINAHL and Google Scholar yielded a total of 1,189 records. After removing duplicates (n=422) and screening titles and abstracts, the full texts of the remaining articles were assessed for eligibility. Based on the pre-defined exclusion criteria, 166 articles were excluded during the full-text screening phase. Additionally, five relevant studies were identified through reference tracking of review articles. A detailed overview of the selection process is presented in the PRISMA flow chart (Figure 1).

### Study characteristics

In total, fourteen studies were included in this systematic review. For the diagnosis of anorexia nervosa, most studies employed the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (4). Exceptions include the studies by Kleiman et al., Armougom et al., and Morita et al., which utilized DSM-4 criteria (15-18). In the study by Mörtl et al., the diagnosis was established based on the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) (19, 20). When studies distinguished between the restrictive and binge-purge subtypes of anorexia nervosa, only data pertaining to the restrictive subtype were included in this review. The majority of the included studies focused exclusively on female patients and healthy female controls, which aligns with the epidemiological

TABLE 2: Alpha and beta diversity in AN (diagnosis) compared to HC.

| Author                  | Publication year | Population (AN/HC) | Stool analysis | Results         |                |
|-------------------------|------------------|--------------------|----------------|-----------------|----------------|
|                         |                  |                    |                | Alpha diversity | Beta diversity |
| Monteleone et al. (22)  | 2020             | 21/20              | 16S PCR        |                 |                |
| Monteleone et al. (21)  | 2021             | 17/20              | 16S PCR        |                 |                |
| Kleiman et al. (15)     | 2015             | 16/12              | 16S PCR        |                 |                |
| Yuan et al. (23)        | 2022             | 30/30              | 16S PCR        |                 |                |
| Schulz et al. (25)      | 2020             | 19/20              | 16S PCR        |                 |                |
| Mörkl et al. (19)       | 2017             | 18/26              | 16S PCR        |                 |                |
| Mack et al. (24)        | 2016             | 55/55              | 16S PCR        |                 |                |
| Borgo et al. (26)       | 2017             | 15/15              | 16S PCR        |                 |                |
| Andreani et al. (27)    | 2024             | 57/34              | 16S PCR        |                 |                |
| Prochazkova et al. (28) | 2021             | 52/67              | 16S PCR        |                 |                |
| Fan et al. (5)          | 2023             | 77/70              | 16S PCR        | /               |                |
| Fouladi et al. (13)     | 2022             | 93/98              | NGS            | /               |                |

| Alpha diversity (CHAO1-index) |                                                                         |
|-------------------------------|-------------------------------------------------------------------------|
|                               | Trend towards higher alpha diversity in AN (diagnosis) compared to HC   |
|                               | No difference reported                                                  |
|                               | Trend towards lower alpha diversity in AN (diagnosis) compared to HC    |
|                               | Alpha diversity is significantly lower in AN (diagnosis) compared to HC |
| Beta diversity                |                                                                         |
|                               | Microbiome composition differs between AN and HC                        |
|                               | Microbiome composition is significantly different between AN and HC     |

pattern of anorexia nervosa. Table 1 summarizes the characteristics of the included studies, including BMI (Body Mass Index) age and illness/treatment duration.

Gut microbiome of AN (diagnosis) compared to HC

The results concerning alpha and beta diversity from twelve included studies are summarized in Table 2. Two studies were excluded from this table due to the absence of explicit reporting on these outcome measures (16, 17). Table 2 is organized according to findings related to alpha-diversity in terms of richness, specifically based on the Chao1 index. This method of organization places the three top-listed studies in the table as those that reported a significantly lower alpha-diversity in patients with anorexia nervosa compared to healthy controls (15, 21, 22). Four studies subsequently reported a trend towards a lower alpha-diversity among patients, while one study reported no difference between the two groups (19, 23-26). Toward the bottom of the table, two studies reported an increase in alpha diversity in patients with anorexia nervosa relative to healthy individuals (27, 28). Finally Table 2 ends with two articles that did not report explicit findings regarding alpha-diversity, but that nonetheless provided relevant data on beta diversity (5, 13). Since the studies were sorted based

on the results of the Chao1 index, beta diversity findings appear in a non-systematic order within the table. Nevertheless, Table 2 shows that in all twelve studies, the composition of the gastrointestinal microbiome (beta diversity) was found to differ – significantly or non-significantly – between patients with anorexia nervosa at the time of diagnosis and healthy controls. In addition, Figure 2 presents a concise summary of the relative abundance of specific microorganisms (families, genera, classes and phyla) in the gut microbiome of patients with anorexia nervosa compared to healthy controls. In line with Table 2, these data refer to the period around the time of diagnosis of the patients with anorexia nervosa. Figure 2 reveals that most of included microorganisms were found in lower abundance in the gut microbiome of patients compared to controls. Notably, all these included microorganisms that showed reduced presence in patients belonged to the Firmicutes phylum. In contrast, a few microorganisms from the Bacteroidota phylum were found in higher abundance in patients relative to healthy individuals. Fan et al. also reported several noteworthy correlations between specific microorganisms and psychological characteristics (5). For example, the absolute abundance of the genus *Parasutterella* was positively correlated with body dissatisfaction. Due to the selection procedure applied

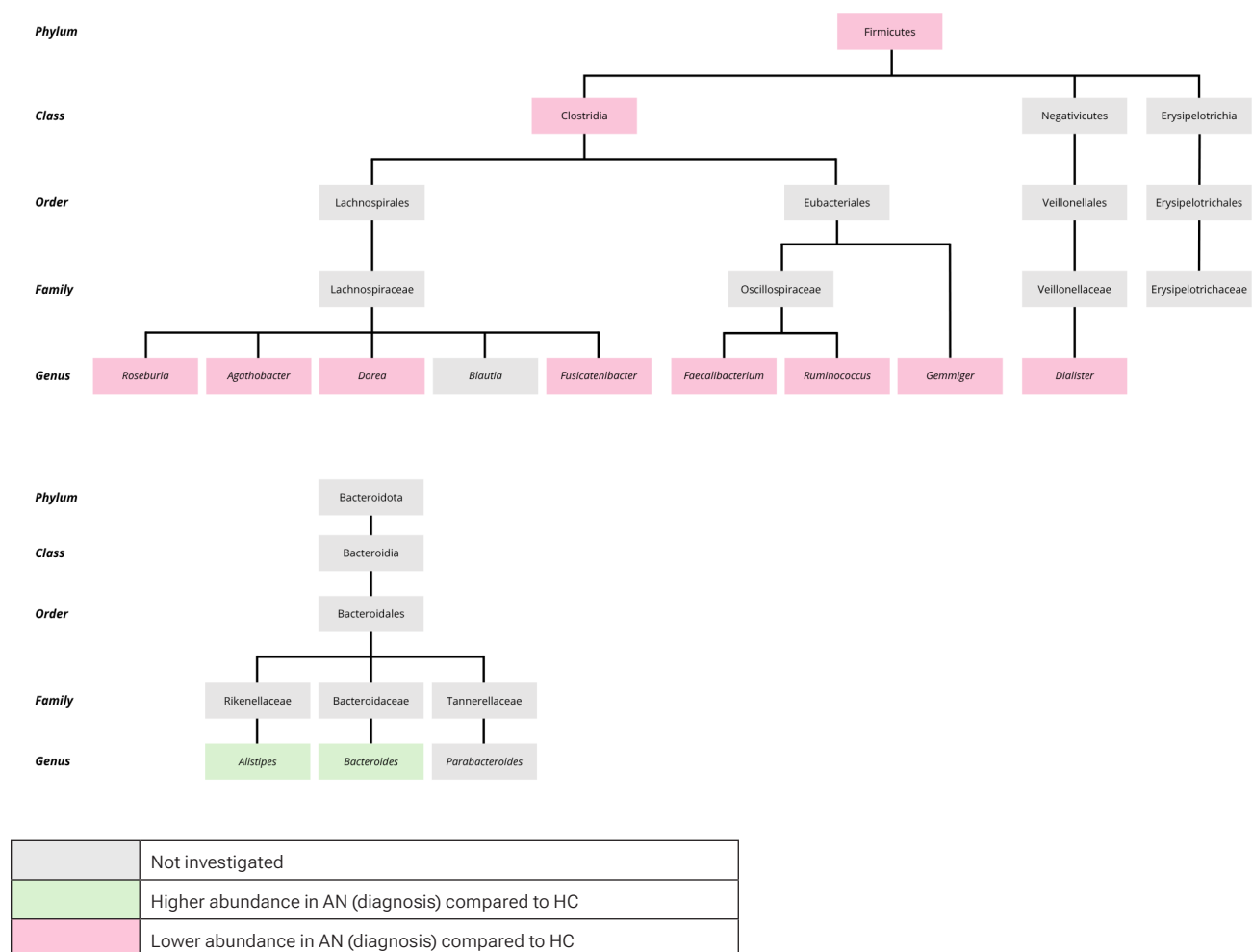


FIGURE 2: Abundances of microorganisms AN (diagnosis) compared to HC.

to determine which microorganisms were included in Figure 2, *Parasutterella* was not included. However, this genus was investigated by Monteleone et al. and Monteleone et al., both of which found a higher abundance of *Parasutterella* in patients with anorexia nervosa at the time of diagnosis compared to healthy controls (21, 22).

#### Gut microbiome of AN (after treatment) compared to AN (diagnosis)

Table 3 includes data from the seven studies that also conducted analyses after a defined treatment period. The structure of the table follows the same logic as Table 2, with studies ordered based on the changes in alpha diversity related to richness (Chao1 index) between the time of diagnosis and after treatment. At the top of Table 3 are two studies reporting a significant increase in alpha diversity of patients after treatment compared to the diversity levels of these patients at the time of diagnosis (24, 25). These are followed by two studies that reported a non-significant increase and two that observed a decrease in alpha diversity post-treatment (15, 22, 27, 28). The table ends with one study that did not provide explicit information on the Chao1 index but still contained relevant data regarding beta diversity

(13). Furthermore, this table also clearly presents the data related to beta diversity. Only one study did not find changes in the composition of the gut microbiome after treatment compared to the gut microbiome at the time of diagnosis (25). Additionally, the study by Prochazkova et al. did not report explicit findings on compositional changes of the gut microbiome in patients with anorexia nervosa (28). The remaining five studies uniformly indicate that the composition of the gut microbiome undergoes changes during the course of treatment (13, 15, 22, 24, 27). Consistent to Figure 2, the data in Figure 3 provides relevant insights into the abundance of specific microorganisms before and after treatment. Figure 3 demonstrated that microorganisms belonging to the phylum Firmicutes are present in higher abundance following treatment, compared to their abundance at diagnosis. In contrast, the abundance of *Alistipes* and *Bacteroides* – both belonging to the phylum Bacteroidota – decreased over the course of treatment.

## Discussion

This systematic review highlights consistent differences in both the richness and composition of the gastrointestinal microbiome between patients with

TABLE 3: Alpha and beta diversity in AN (after treatment,  $t_1$ ) compared to AN (diagnosis,  $t_0$ ).

| Author                  | Publication year | Population AN ( $t_0/t_1$ ) | Stool analysis | Results         |                |
|-------------------------|------------------|-----------------------------|----------------|-----------------|----------------|
|                         |                  |                             |                | Alpha diversity | Beta diversity |
| Schulz et al. (25)      | 2020             | 19/19                       | 16S            |                 |                |
| Mack et al. (24)        | 2016             | 55/44                       | 16S            |                 |                |
| Monteleone et al. (22)  | 2020             | 21/16                       | 16S            |                 |                |
| Kleiman et al. (15)     | 2015             | 16/10                       | 16S            |                 |                |
| Andreani et al. (27)    | 2024             | 57/50                       | 16S            |                 |                |
| Prochazkova et al. (28) | 2021             | 52/52                       | 16S            |                 | /              |
| Fouladi et al. (13)     | 2022             | 93/78                       | NGS            | /               |                |

| Alpha diversity (CHAO1-index) |                                                                                            |
|-------------------------------|--------------------------------------------------------------------------------------------|
|                               | Alpha diversity increased significantly in AN (after treatment) compared to AN (diagnosis) |
|                               | Trend towards higher alpha diversity in AN (after treatment) compared to AN (diagnosis)    |
|                               | Trend towards lower alpha diversity in AN (after treatment) compared to AN (diagnosis)     |

| Beta diversity |                                                                                                                  |
|----------------|------------------------------------------------------------------------------------------------------------------|
|                | The difference in microbiome composition between AN and HC decreases after treatment, but does not fully recover |
|                | The microbiome composition remains equally different between AN and HC after treatment                           |

anorexia nervosa and healthy individuals, as well as within the patient group. This raises the question of whether well-founded hypotheses can be developed concerning the abundance of specific microorganisms in relation to the stage of illness and the treatment trajectory in patients with anorexia nervosa.

### Gut microbiome at the time of diagnosis

The included studies show that the alpha diversity based on richness (Chao1 index) of the gastrointestinal microbiome is generally lower in patients with anorexia nervosa at the time of diagnosis compared to healthy individuals. Furthermore, this review indicated that the composition (beta diversity) of the microbiome also differs – either significantly or non-significantly – between patients at diagnosis and healthy controls. These microbiome alterations are also reflected in the analysis of the abundance of various microbial taxa. In addition, the findings suggest a potential correlation between a reduced abundance of microorganisms belonging to the phylum Firmicutes and anorexia nervosa, particularly at the time of diagnosis. Conversely, microorganisms belonging to the phylum Bacteroidota appear to be more abundant in patients compared to healthy controls. This suggests a possible association between Bacteroidota abundance and anorexia nervosa. Moreover, two studies report an increased presence of the genus *Parasutterella* in patients with anorexia nervosa relative to healthy individuals (21, 22). This microorganism has been linked to body dissatisfaction (5). Given its elevated abundance in patients with anorexia nervosa, *Parasutterella* might be hypothesized to play a role in disturbances in body image perception, which may have implications for the recovery process.

### Gut microbiome after treatment

Comparing the composition and richness of the gastrointestinal microbiome in patients at the time of diagnosis with analyses conducted after treatment yields several noteworthy findings. Primarily, the majority of included studies report an increase in alpha diversity, as well as a shift in the microbiome composition (beta diversity) towards a profile more closely resembling that of healthy individuals. However, no study demonstrated that the microbiome composition of patients post-treatment becomes fully comparable to that of the healthy control group. Changes in the gastrointestinal microbiome composition following treatment are also reflected in the abundance analysis of specific microorganisms. These analyses show that the genera *Alistipes*, *Bacteroides* and *Parabacteroides*, as well as the phylum Bacteroidota, are less abundant in the microbiome of the patients after treatment. In line with the hypothesis suggested above – that microorganisms belonging to the phylum Bacteroidota may be associated with anorexia nervosa – these microorganisms are indeed found in reduced abundance after treatment and subsequent weight gain in patients. These findings suggest a potential relationship between microorganisms of the order Bacteroidales and the health and weight status of patients with anorexia nervosa. Furthermore, this study shows that microorganisms belonging to the phylum Firmicutes are less abundant in the gastrointestinal microbiome of these patients at diagnosis. After treatment, the abundance of these microorganisms increases. These observations also support a possible correlation between microbial composition and the nutritional and weight status of patients.

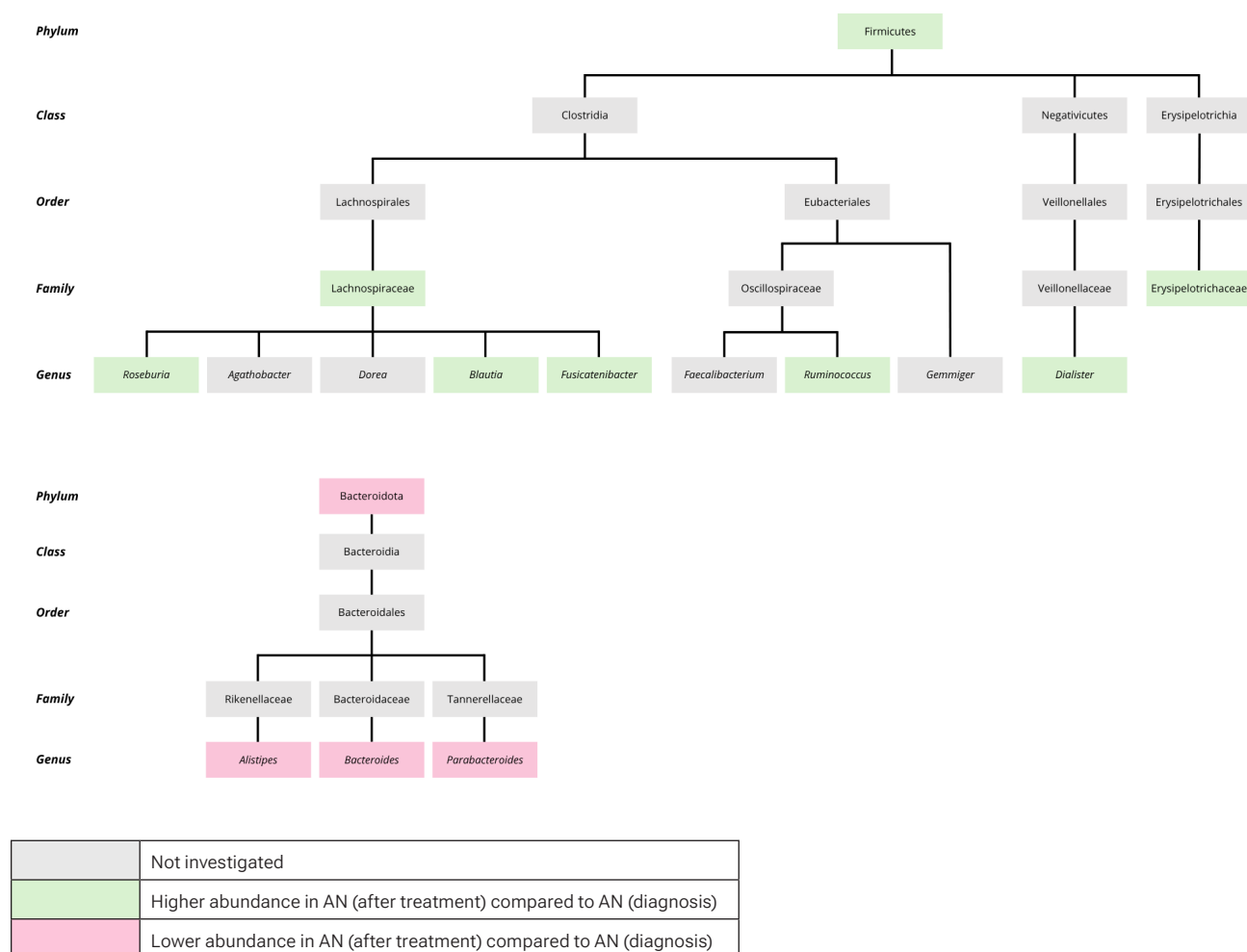


FIGURE 3: Abundance of microorganisms in AN (after treatment,  $t_1$ ) compared to AN (diagnosis,  $t_0$ ).

## Key insights

The study conducted by Menni et al., investigating a population of healthy women, reports an association between the families Oscillospiraceae and Lachnospiraceae and reduced long-term weight-gain, whereas the genus *Bacteroides* is linked to an increased risk of weight gain (29). When these findings are considered alongside the results of this literature review regarding the microbiome at diagnosis, a possible connection emerges. This review reports an elevated abundance of *Bacteroides* in patients with anorexia nervosa at the time of diagnosis. Given that *Bacteroides* has been associated with a higher risk of weight gain, it is plausible that this increased presence reflects a physiological response aimed at promoting weight gain in response to undernutrition. Conversely, several genera within the families Oscillospiraceae and Lachnospiraceae are found to be reduced at diagnosis, which might similarly be explained as a protective mechanism of the body in response to the underweight status. These hypotheses are further supported by the study of de Clercq et al., which demonstrated that faecal microbiota transplantation with a high abundance of the phylum Bacteroidota and a lower abundance of Firmicutes resulted in significant weight gain (30).

After treatment, when patients typically exhibit less severe underweight, a decreased abundance of microorganisms belonging to the phylum Bacteroidota and an increased abundance of those belonging to Firmicutes are reported. These findings could also be interpreted within the framework of the aforementioned hypothesis, suggesting that at this stage, the body may have less need to actively stimulate weight gain. When comparing the findings of this systematic review with recent data from healthy individuals, the study by Yamamoto-Wada et al. provides crucial insights (31). This study, which excluded individuals with underlying diseases, demonstrated that the gut microbiota of underweight women (BMI < 17.5) differs significantly from that of normal-weight controls. Specifically, the underweight group exhibited a significantly lower alpha diversity. Although it should be noted that Yamamoto-Wada et al. utilized different diversity metrics than the Chao1 index employed in the current review – precluding a direct statistical comparison – the overarching trend consistently points toward reduced alpha diversity in low-BMI populations. Furthermore, the underweight group in their study showed a decreased abundance of taxa such as *Dorea*, *Fusicatenibacter* and *Agathobacter*. This aligns with the lower abundance of these bacteria observed

in patients with anorexia nervosa at the time of diagnoses, as discussed previously. These results allow us to cautiously suggest that certain microbial alterations are indeed linked to low body weight itself.

### Strengths and limitations

A key strength of this systematic review is the comparison of findings from fourteen individual studies, providing a broader and deeper understanding of the gastrointestinal microbiome's composition and dynamics in patients with anorexia nervosa. This collective approach enhances the relevance of the findings and increases the likelihood of identifying consistent patterns, enabling better-supported hypotheses and informing future clinical research. However, several limitations should be considered when interpreting the results. The selection of the fourteen articles was based on strict exclusion criteria, which may have omitted relevant studies. For example, non-English publications were excluded, potentially overlooking important data from countries with different dietary cultures that could influence microbiome composition and diversity. Furthermore, animal studies were excluded, possibly missing valuable mechanistic insights. Additionally, the included studies vary in methodology, timing of sample collection, exclusion criteria, and potentially participants' diets, limiting comparability and reducing the overall reliability of combined conclusions. Most studies focused exclusively on female patients and controls, leaving possible sex differences underexplored. Only one study examined long-term microbiome changes, restricting conclusions about sustained effects. Finally, two studies – Andreani et al. and Prochazkova et al. – reported findings on alpha diversity at diagnosis and after treatment that contradict the other studies (27, 28). This may indicate the influence of other confounding factors, although no clear differences explaining these discrepancies were identified.

### Clinical implementation

An underexplored aspect in current research on the gastrointestinal microbiome in patients with anorexia nervosa is the potential interaction between different types of food and the abundance of specific microorganisms. Scientific studies suggest a shift in microbial abundance in a direction that, based on the aforementioned hypotheses, would be favourable to promote weight gain in these patients when following a protein-rich or fat-rich diet (32-34). Conversely, a carbohydrate-rich diet might potentially limit weight gain (35). Considering that standard hypercaloric nutritional supplementation derives approximately half of its energy from carbohydrates, it could be suggested that this composition may not be optimal for stimulating weight gain in this patient population when taking the suggested microbiome's response into account (36). Regarding the effect of enteral nutrition on the gastrointestinal microbiome, no definitive conclusions can

yet be drawn, as studies in patients with Crohn's disease have produced conflicting results (37). However, one study suggests that industrially prepared enteral nutrition may be favourable for promoting weight gain than blended diet when considering the microbiome's impact (38). A critical examination of the impact of various kinds of nutrition – whether standard diet, medical supplementation or enteral feeding – on the microbiome could provide valuable insights. A better understanding of the interplay between different types of food or nutrients and the gastrointestinal microbiome, combined with the aforementioned hypotheses, could contribute to more targeted treatment strategies and potentially shorten the recovery process.

### Conclusion

This systematic review examined the relationship between the gastrointestinal microbiome and anorexia nervosa. The included studies indicate that patients with anorexia nervosa exhibit, at diagnosis, reduced microbial richness (Chao1 index, alpha diversity) and an altered composition (beta diversity) of the gastrointestinal microbiome compared to healthy individuals. Consistent with these findings, clear differences were also reported in the abundance of various microorganisms. Furthermore, this review shows that both the richness and composition of the microbiome undergo significant changes throughout treatment. Generally, this results in increased microbial richness and shifts in composition. However, these changes do not lead to a full normalization of the gastrointestinal microbiome toward the profile observed in healthy individuals. Additionally, Fan et al. demonstrated associations between specific microorganisms and psychological traits such as body dissatisfaction and the drive for thinness (5). Moreover, scientific research points to a potential influence of the gastrointestinal microbiome composition on weight evolution (29, 30). This review highlights the importance of the gastrointestinal microbiome as a promising yet underexplored factor in the treatment of anorexia nervosa. The observed microbial differences and their changes during treatment suggest a possible link to weight gain. Focused research on this relationship and intervention possibilities through nutrition could represent a crucial step toward improving therapeutic approaches.

### COI statement

All authors certify that they have no conflicts of interest (COIs), including financial or other relationships with the product or its manufacturer. Moreover, the manufacturer had no influence on the design, methodology or reporting of this review.

# Supplementary file: search strategy

## PUBMED

### Subject 1

("Feeding and Eating disorders"[MeSH] OR "Eating disorders"[tiab] OR "Eating disorder"[tiab] OR "Pathologic Eating Behavior"[tiab] OR "Disordered Eating Behavior"[tiab] OR "Disordered Eating Behaviour"[tiab] OR "Disordered Eating"[tiab] OR "Anorexia Nervosa"[MeSH] OR "Anorexia"[tiab] OR "Anorexia Nervosa"[tiab] OR "Binge-Eating Disorder"[MeSH] OR "Binge-Eating Disorder"[tiab] OR "Binge eating syndrome"[tiab] OR "Binge-eating syndrome"[tiab] OR "Bulimia Nervosa"[MeSH] OR "Bulimia"[tiab] OR "Bulimia Nervosa"[tiab])

### Subject 2

"Gastrointestinal Microbiome"[MeSH] OR "Gastrointestinal Microbiome"[tiab] OR "Gastrointestinal Flora"[tiab] OR "Gastrointestinal Microflora"[tiab] OR "Gut Microbiome"[tiab] OR "Gut Microflora"[tiab] OR "Gut Microbiota"[tiab] OR "Gut bacteria"[tiab] OR "Intestinal Flora"[tiab] OR "Intestine Flora"[tiab] OR "Intestinal Microbiome"[tiab] OR "Intestinal Microbiota"[tiab] OR "Intestinal Microflora"[tiab] OR "bowel microbiota"[tiab] OR "Gastro Intestinal Flora"[tiab] OR "Gastrointestinal Microbiota"[tiab] OR "gastrointestinal tract flora"[tiab] OR "Gastrointestine Flora"[tiab] OR "Gastrointestine tract flora"[tiab] OR "Intestinal bacteria"[tiab] OR "Intestinal Bacterial Flora"[tiab] OR "Intestinal Canal Flora"[tiab] OR "Intestine Microbial Flora"[tiab] OR "Intestine Microflora"[tiab]

### Combination:

(subject 1) AND (subject 2)

("Feeding and Eating disorders"[MeSH] OR "Eating disorders"[tiab] OR "Eating disorder"[tiab] OR "Pathologic Eating Behavior"[tiab] OR "Disordered Eating Behavior"[tiab] OR "Disordered Eating Behaviour"[tiab] OR "Disordered Eating"[tiab] OR "Anorexia Nervosa"[MeSH] OR "Anorexia"[tiab] OR "Anorexia Nervosa"[tiab] OR "Binge-Eating Disorder"[MeSH] OR "Binge-Eating Disorder"[tiab] OR "Binge eating syndrome"[tiab] OR "Binge-eating syndrome"[tiab] OR "Bulimia Nervosa"[MeSH] OR "Bulimia"[tiab] OR "Bulimia Nervosa"[tiab]) AND ("Gastrointestinal Microbiome"[MeSH] OR "Gastrointestinal Microbiome"[tiab] OR "Gastrointestinal Flora"[tiab] OR "Gastrointestinal Microflora"[tiab] OR "Gut Microbiome"[tiab] OR "Gut Microflora"[tiab] OR "Gut Microbiota"[tiab] OR "Gut bacteria"[tiab] OR "Intestinal Flora"[tiab] OR "Intestine Flora"[tiab] OR "Intestinal Microbiome"[tiab] OR "Intestinal Microbiota"[tiab] OR "Intestinal Microflora"[tiab] OR "bowel microbiota"[tiab] OR "Gastro Intestinal Flora"[tiab] OR

"Gastrointestinal Microbiota"[tiab] OR "gastrointestinal tract flora"[tiab] OR "Gastrointestine Flora"[tiab] OR "Gastrointestine tract flora"[tiab] OR "Intestinal bacteria"[tiab] OR "Intestinal Bacterial Flora"[tiab] OR "Intestinal Canal Flora"[tiab] OR "Intestine Microbial Flora"[tiab] OR "Intestine Microflora"[tiab])

## EMBASE

### Subject 1

'eating disorder'/exp OR 'eating disorder':ti,ab,kw OR 'eating disorders':ti,ab,kw OR 'pathologic eating behavior':ti,ab,kw OR 'pathologic eating behaviour':ti,ab,kw OR 'disordered eating behaviour':ti,ab,kw OR 'disordered eating behavior':ti,ab,kw OR 'disordered eating':ti,ab,kw OR 'anorexia nervosa'/exp OR 'anorexia nervosa':ti,ab,kw OR 'anorexia':ti,ab,kw OR 'binge eating disorder'/exp OR 'binge eating disorder':ti,ab,kw OR 'binge eating syndrome':ti,ab,kw OR 'binge-eating syndrome':ti,ab,kw OR 'binge-eating disorder':ti,ab,kw OR 'bulimia'/exp OR 'bulimia':ti,ab,kw OR 'bulimia nervosa':ti,ab,kw

### Subject 2

'intestine flora'/exp OR 'intestine flora':ti,ab,kw OR 'bowel microbiota':ti,ab,kw OR 'gastrointestinal flora':ti,ab,kw OR 'gastro intestinal flora':ti,ab,kw OR 'gastrointestinal microbiome':ti,ab,kw OR 'gastrointestinal microbiota':ti,ab,kw OR 'gastrointestinal tract flora':ti,ab,kw OR 'gastrointestine flora':ti,ab,kw OR 'gastrointestine tract flora':ti,ab,kw OR 'gut bacteria':ti,ab,kw OR 'gut microbiota':ti,ab,kw OR 'intestinal bacteria':ti,ab,kw OR 'intestinal bacterial flora':ti,ab,kw OR 'intestinal flora':ti,ab,kw OR 'intestinal canal flora':ti,ab,kw OR 'intestinal microbiota':ti,ab,kw OR 'intestinal microflora':ti,ab,kw OR 'intestine microbial flora':ti,ab,kw OR 'intestine microflora':ti,ab,kw OR 'intestine flora':ti,ab,kw

### Combination:

(subject 1) AND (subject 2)

('eating disorder'/exp OR 'eating disorder':ti,ab,kw OR 'eating disorders':ti,ab,kw OR 'pathologic eating behavior':ti,ab,kw OR 'pathologic eating behaviour':ti,ab,kw OR 'disordered eating behaviour':ti,ab,kw OR 'disordered eating behavior':ti,ab,kw OR 'disordered eating':ti,ab,kw OR 'anorexia nervosa'/exp OR 'anorexia nervosa':ti,ab,kw OR 'anorexia':ti,ab,kw OR 'binge eating disorder'/exp OR 'binge eating disorder':ti,ab,kw OR 'binge eating syndrome':ti,ab,kw OR 'binge-eating syndrome':ti,ab,kw OR 'binge-eating disorder':ti,ab,kw OR 'bulimia'/exp OR 'bulimia':ti,ab,kw OR 'bulimia nervosa':ti,ab,kw) AND ('intestine flora'/exp OR 'intestine flora':ti,ab,kw OR 'bowel microbiota':ti,ab,kw OR



1. Silen Y, Keski-Rahkonen A. Worldwide prevalence of DSM-5 eating disorders among young people. *Curr Opin Psychiatry*. 2022;35(6):362-71.
2. Sciensano. Gewichtstoestand en eetstoornissen: Eetstoornissen, Voedselconsumptiepeiling 2022-2023. Brussels, Belgium: Sciensano; 2024 [cited 2025 April 3]. Available from: <https://www.sciensano.be/nl/resultaten-voedselconsumptiepeiling-2022-2023/gewichtstoestand-en-eetstoornissen/eetstoornissen>.
3. Auger N, Potter BJ, Ukah UV, Low N, Israel M, Steiger H, et al. Anorexia nervosa and the long-term risk of mortality in women. *World Psychiatry*. 2021;20(3):448-9.
4. Diagnostic and Statistical Manual of Mental Disorders - DSM-5. Fifth ed. Arlington, Virginia, USA: American Psychiatric Association; 2013.
5. Fan Y, Stoving RK, Berreira Ibraim S, Hyotylainen T, Thirion F, Arora T, et al. The gut microbiota contributes to the pathogenesis of anorexia nervosa in humans and mice. *Nat Microbiol*. 2023;8(5):787-802.
6. Thursby E, Juge N. Introduction to the human gut microbiota. *Biochem J*. 2017;474(11):1823-36.
7. Naveed M, Zhou QG, Xu C, Taleb A, Meng F, Ahmed B, et al. Gut-brain axis: A matter of concern in neuropsychiatric disorders...! *Prog Neuropsychopharmacol Biol Psychiatry*. 2021;104:110051.
8. Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CS. The gut microbiota in anxiety and depression—a systematic review. *Clinical psychology review*. 2021;83:101943.
9. Alpha Diversity. Seoul, South Korea: CJ Bioscience Inc., EzBioCloud Knowledge Base; [cited 2025 April 4]. Available from: <https://kb.ezbiocloud.net/home/science-blogs/analyze/alpha-diversity>.
10. Hagerty SL, Hutchison KE, Lowry CA, Bryan AD. An empirically derived method for measuring human gut microbiome alpha diversity: Demonstrated utility in predicting health-related outcomes among a human clinical sample. *PLoS One*. 2020;15(3):e0229204.
11. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
12. Polymerase Chain Reaction (PCR). Bethesda, Maryland, USA: National Library of Medicine, National Center for Biotechnology Information; [cited 2025 April 4]. Available from: <https://www.ncbi.nlm.nih.gov>.
13. Fouladi F, Bulik-Sullivan EC, Glennly EM, Thornton LM, Reed KK, Thomas S, et al. Reproducible changes in the anorexia nervosa gut microbiota following inpatient therapy remain distinct from non-eating disorder controls. *Gut Microbes*. 2022;14(1):2143217.
14. Sharpton TJ. An introduction to the analysis of shotgun metagenomic data. *Front Plant Sci*. 2014;5:209.
15. Kleiman SC, Watson HJ, Bulik-Sullivan EC, Huh EY, Tarantino LM, Bulik CM, et al. The Intestinal Microbiota in Acute Anorexia Nervosa and During Renourishment: Relationship to Depression, Anxiety, and Eating Disorder Psychopathology. *Psychosom Med*. 2015;77(9):969-81.
16. Armougoum F, Henry M, Vialettes B, Raccach D, Raoult D. Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and *Methanogens* in anorexic patients. *PLoS One*. 2009;4(9):e7125.
17. Morita C, Tsuji H, Hata T, Gondo M, Takakura S, Kawai K, et al. Gut Dysbiosis in Patients with Anorexia Nervosa. *PLoS One*. 2015;10(12):e0145274.
18. Diagnostic and Statistical Manual of Mental Disorders - Text Revision (DSM-IV-TR). Fourth ed. Arlington, Virginia, USA: American Psychiatric Association; 2000.
19. Morkl S, Lackner S, Muller W, Gorkiewicz G, Kashofer K, Oberascher A, et al. Gut microbiota and body composition in anorexia nervosa inpatients in comparison to athletes, overweight, obese, and normal weight controls. *Int J Eat Disord*. 2017;50(12):1421-31.
20. International statistical classification of diseases and related health problems - 10th revision (ICD-10). Geneva, Switzerland: World Health Organization; 2016 [cited 2025 April 4]. Available from: <https://iris.who.int/items/5858156c-f66b-43cc-be2c-37710e5cd2e6>.
21. Monteleone AM, Troisi J, Serena G, Fasano A, Dalle Grave R, Cascino G, et al. The Gut Microbiome and Metabolomics Profiles of Restricting and Binge-Purging Type Anorexia Nervosa. *Nutrients*. 2021;13(2).
22. Monteleone AM, Troisi J, Fasano A, Dalle Grave R, Marciello F, Serena G, et al. Multi-omics data integration in anorexia nervosa patients before and after weight regain: A microbiome-metabolomics investigation. *Clin Nutr*. 2021;40(3):1137-46.
23. Yuan R, Yang L, Yao G, Geng S, Ge Q, Bo S, et al. Features of gut microbiota in patients with anorexia nervosa. *Chin Med J (Engl)*. 2022;135(16):1993-2002.
24. Mack I, Cuntz U, Gramer C, Niederauer S, Pohl C, Schwietz A, et al. Weight gain in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid profiles, and gastrointestinal complaints. *Sci Rep*. 2016;6:26752.
25. Schulz N, Belheouane M, Dahmen B, Ruan VA, Specht HE, Dempfle A, et al. Gut microbiota alteration in adolescent anorexia nervosa does not normalize with short-term weight restoration. *Int J Eat Disord*. 2021;54(6):969-80.
26. Borgo F, Riva A, Benetti A, Casiraghi MC, Bertelli S, Garbossa S, et al. Microbiota in anorexia nervosa: the triangle between bacterial species, metabolites and psychological tests. *PLoS one*. 2017;12(6):e0179739.
27. Andreani NA, Sharma A, Dahmen B, Specht HE, Mannig N, Ruan V, et al. Longitudinal analysis of the gut microbiome in adolescent patients with anorexia nervosa: microbiome-related factors associated with clinical outcome. *Gut Microbes*. 2024;16(1):2304158.
28. Prochazkova P, Roubalova R, Dvorak J, Kreisinger J, Hill M, Tlaskalova-Hogenova H, et al. The intestinal microbiota and metabolites in patients with anorexia nervosa. *Gut Microbes*. 2021;13(1):1-25.
29. Menni C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM. Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. *Int J Obes (Lond)*. 2017;41(7):1099-105.
30. de Clercq NC, Frissen MN, Davids M, Groen AK, Nieuwdorp M. Weight Gain after Fecal Microbiota Transplantation in a Patient with Recurrent Underweight following Clinical Recovery from Anorexia Nervosa. *Psychother Psychosom*. 2019;88(1):58-60.
31. Yamamoto-Wada R, Hiraiwa E, Okuma K, Yamada M, Ushiroda C, Deguchi K, et al. Gut Microbiota alpha- and beta-Diversity, but Not Dietary Patterns, Differ Between Underweight and Normal-Weight Japanese Women Aged 20-39 Years. *Nutrients*. 2025;17(20).
32. Kim E, Kim DB, Park JY. Changes of Mouse Gut Microbiota Diversity and Composition by Modulating Dietary Protein and Carbohydrate Contents: A Pilot Study. *Prev Nutr Food Sci*. 2016;21(1):57-61.
33. Daniel H, Gholami AM, Berry D, Desmarchelier C, Hahne H, Loh G, et al. High-fat diet alters gut microbiota physiology in mice. *ISME J*. 2014;8(2):295-308.
34. Wan Y, Wang F, Yuan J, Li J, Jiang D, Zhang J, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. *Gut*. 2019;68(8):1417-29.
35. Li L, Zhao X, Abudugheni R, Yu F, Zhao Y, Ma BF, et al. Gut microbiota changes associated with low-carbohydrate diet intervention for obesity. *Open Life Sci*. 2024;19(1):20220803.
36. Fortimel 1.5 kcal.: Nutricia; [cited 2025 May 3]. Available from: <https://www.nutricia.be/producten/Fortimel-1-5-kcal.html>.
37. Horwat P, Kopec S, Garczyk A, Kaliciak I, Starega Z, Drogowski K, et al. Influence of Enteral Nutrition on Gut Microbiota Composition in Patients with Crohn's Disease: A Systematic Review. *Nutrients*. 2020;12(9).
38. Gallagher K, Flint A, Mouzaki M, Carpenter A, Haliburton B, Bannister L, et al. Blenderized Enteral Nutrition Diet Study: Feasibility, Clinical, and Microbiome Outcomes of Providing Blenderized Feeds Through a Gastric Tube in a Medically Complex Pediatric Population. *JPEN J Parenter Enteral Nutr*. 2018;42(6):1046-60.