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Gut microbiota and intestinal barrier-related markers in patients with anorexia nervosa: Systematic review

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Summary

The aim of this systematic review was to determine: (1) differences between patients with anorexia nervosa (AN) and healthy controls (HC) in terms of gut microbiota community and intestinal barrier-related markers; (2) relationship between the intestinal ecosystem and health-related factors in AN individuals. We conducted a systematic literature search (PubMed, Embase, ClinicalTrials registry) until 30 September 2020 for studies reporting gut microbiome and intestinal barrier-related markers in patients with AN. Six studies on intestinal microbiota were eligible for this review, including three papers also describing intestinal barrier markers. Among five studies analyzing microbiota diversity, four of them found differences between AN patients and HC. The studies confirm alterations of the markers of the intestinal barrier integrity in patients with anorexia. The systematic review confirms changes in the gut ecosystem of patients with an eating disorder, without a clear consensus of microbiota patterns in AN. Damage of intestinal barrier integrity is poorly documented in AN patients and needs more attention in further studies.

Key words: gut microbiome, gut-brain axis, anorexia nervosa

Introduction

According to ICD-11, anorexia nervosa (AN) is an eating disorder (ED) characterized by an inadequate (too low) body mass index (BMI), fear of gaining weight, lack of motivation or ambivalence toward change, and striving for self-control. AN patients

focus on behavior patterns that prevent the restoration of healthy body weight (qualitative and quantitative food restrictions, regular and excessive physical activity, use of laxatives) [1]. AN leads to serious health consequences, and the risk of mortality in this population is 10% greater compared to healthy persons [2]. A proposed therapeutic target in ED is the gut microbiota. There is a growing interest in the crosstalk between the gut ecosystem and the brain, known as the microbiome-gut-brain axis [3, 4].

The gastrointestinal tract produces numerous neuroactive peptides, both orexigenic (appetite-stimulating) and anorexigenic (appetite-suppressing). The balance between these neuropeptides plays an important role in the regulation of food intake, and the control center of this process is the hypothalamus [5]. Microorganisms produce various metabolites that affect the host metabolism via gut mucosa interaction. The most studied compounds are short-chain fatty acids (SCFAs) but cytokines, neuro – and stress-related hormones (serotonin, dopamine, norepinephrine, cortisol, adrenocorticotropic hormone) are synthesized by the gut microbiota as well [6]. In the molecular mimicry concept, antigens (e.g., caseinolytic peptidase B protein homolog – ClpB protein) produced by microbiota have the potential for cross-reactivity with alpha-melanocyte-stimulating hormone (alpha-MSH). Production of antibodies against bacterial proteins is associated with decreased food intake, weight loss and increased anxiety [4].

The altered gut composition strongly affects intestinal barrier function and consequently its permeability. Chronic food restriction and starvation enhance impairment of the gut mucosa and together with higher levels of mucin-degrading bacteria trigger an immune-inflammatory response [3]. Thus, manipulations of microbiota composition could have a promising role in the therapy of anorexia nervosa. Nevertheless, to date, there has been no comprehensive analysis determining the gut barrier dysfunction and gut microbiota pattern in patients with an eating disorder.

Therefore, we performed a systematic review of available literature to investigate: (1) differences in alterations of the gut microbiota community and intestinal barrier marker levels between AN patients and healthy controls (HC), (2) the relationship between the intestinal ecosystem and health-related factors in patients suffering from AN.

Materials and methods

Search strategy and inclusion criteria

Two authors (JR and OP) independently and systematically searched PubMed/Embase/ClinicalTrials Registry from the inception of the study until 30.09.2020 for original papers on the gut microbiota and intestinal barrier in patients suffering from AN. The following search strings were used: (1) PubMed – Microbiota OR Gastrointestinal Microbiome OR microbiome OR Acetates OR Butyrates OR Butyric Acid OR Propionates OR SCFA OR Toxins, Biological OR Bacterial Toxins OR Endotoxins OR Lipopolysaccharides OR Antigens OR LPS OR LPBP OR lypopolysaccharide binding protein OR FABP OR fatty acid binding protein OR zonulin OR calprotectin OR faecal alpha-1-antitrypsin OR intestinal barrier OR gut barrier AND Anorexia nervosa; (2)

Embase – anorexia nervosa/exp AND microflora/exp OR microbial flora OR microbiota OR microflora OR microbiome/exp OR microbiome OR microbiomes OR short chain fatty acids OR lipopolysaccharide/exp OR lipopolysaccharide OR lipopolysaccharide b OR lipopolysaccharides OR lps OR fatty acid binding protein/exp OR fatty acid binding protein 2/exp OR fabp2 protein OR fatty acid binding protein 2 OR intestinal fatty acid binding protein OR protein fabp2 OR zonulin/exp OR calprotectin OR gut barrier/exp OR intestinal barrier/exp); (3) ClinicalTrials – Anorexia nervosa, Microbiome.

The electronic search was followed by a manual screen of the reference lists from eligible publications. The inclusion criteria were:

(1) Anorexia nervosa clinical diagnosis, according ICD-10 criteria [7].
(2) BMI less than or equal to 17.5 kg/m^2.

The exclusion criteria were:

(1) Presence of any of the following conditions: inflammatory bowel diseases, autoimmune disorders, psychotic disorders, bipolar disorder, autism spectrum disorders, hyperkinetic disorders, immunocompromised disorders, diabetes mellitus, celiac disease, cancer cachexia.
(2) Antibiotics taken within two months before the examination.
(3) Use of probiotics within three months prior to the investigation.

Data extraction

The assessment was performed independently by at least two authors (KSŻ, JR, OP). One author (HKJ) was involved as a dispute referee if a discrepancy appeared. The standard data extraction sheet described in previous studies was used [8, 9]. From each article, we extracted information including details on the study design (type of study, its setting, the focus of the study, outcomes, relationships with clinical variables, methods, conclusions), and the study population (number of participants, sample characteristics). Due to the variety of microbiota-related parameters, we were not able to perform a meta-analysis. Instead, we made an attempt to link the gut microbiota and intestinal barrier disruptions with clinical characteristics of AN.

For evaluation of the risk of bias (ROB), the STROBE Statement was applied, with the exception of item 16 (as this was not assessed in any of the surveyed studies) [10]. When the number of points was below 16 (50% of the maximum STROBE score), the quality was arbitrarily defined as low. A score up to 19 points (60%) meant that the study had moderate quality, and with a score up to or over 23 points (75%), we treated the study as of high or very high quality, respectively.

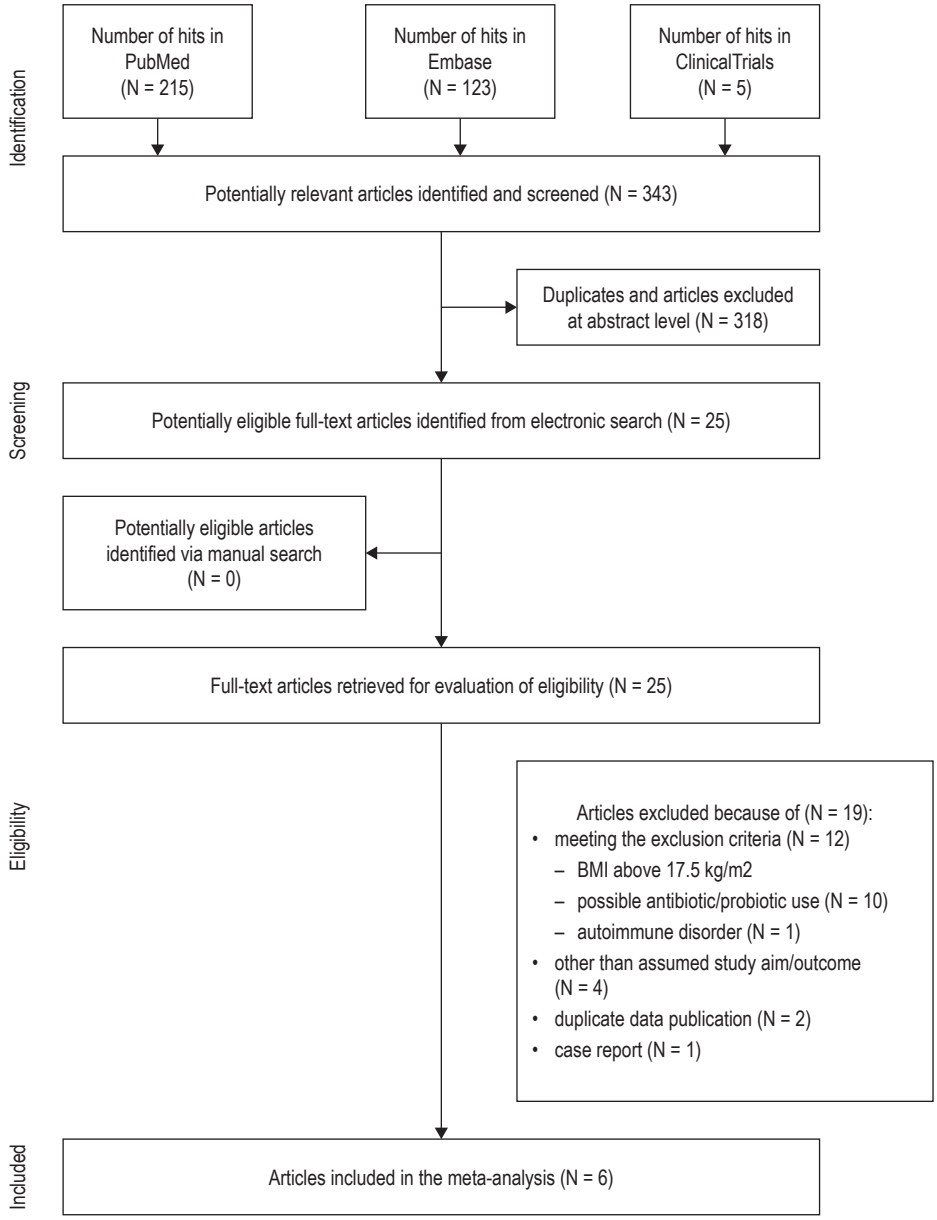

Figure 1. **Study flow chart**

Results

Descriptive data

The initial search yielded 343 hits (see Figure 1). Of these, 318 were excluded as duplicates or after screening the title and/or abstract. Twenty-five articles were subjected for full-text review. Nineteen of them were rejected because they met the exclusion criteria (n = 12), had study aims and/or outcomes other than those of interest (n = 4), or did not fulfill the following inclusion criteria: BMI values other than those determined by the authors of this review (n = 3), possible probiotic/antibiotic use during the three months preceding the study (n = 10), and an autoimmune disorder (n = 1). Additionally, one of the papers was a case report, and two were duplicate publications.

Study and sample characteristics

Participants

Finally, six papers (four case-control studies, one cross-sectional study, and one study whose design was not clearly defined) met the inclusion criteria, with 295 participants: 105 with AN and 190 healthy controls (HC), who were included in the review [11-16]. Of these, three studies were from Europe (two from France and one from Italy), two were from Japan and one was from the USA (see Table 1). A gut microbiome analysis was conducted among 99 participants with AN and 184 from the HC groups (283 individuals in total). Inpatients were included in three analyses, while both in – and outpatients in two analyses.

The sample size of the studies was relatively small, ranging from 3 to 33 persons in AN and from 10 to 91 in the HC groups. The mean BMI ranged from 11.7+/-1.5 kg/m^2 to 14.1+/-1 kg/m^2 in AN groups and from 20.5+/-2.1 kg/m^2 to 22+/-3 kg/m^2 in healthy individuals. Two studies did not mention the BMI of the examined individuals [13, 14].

Table 1. **Study characteristics**

	Study description	Study characteristics				Number of patients		Sample characteristics		
	Reference	Type of study	Setting	Focus of study	ROB	N total included AN/HC	N total analyzed AN/HC	Age (years, mean)±SD (AN/HC)	Males (n) (AN/HC)	Males (%) (AN/HC)
1.	Hanachi et al., 2019 France	case-control	inpatient	the link between microbiota and functional gastrointestinal disorders (Francis score) and clinical and biological parameters of AN	23	33/22	33/22	32±12/36±10	0/1	0/4.5
2.	Hata et al., 2019 Japan	case-control	in – and outpatient	to assess the impact of microbiota transplantation on weight gain and behavioral characteristics	18	10/10	4/4	23±3.4/25.3±0,8	0/0	0/0
3.	Morita et al., 2015 Japan	cross-sectional	in – and outpatient	to examine the link between fecal microbiota and different subtypes of AN	17	25*/21	25*/21	30±10.2/31.5±7.4	0/0	0/0
4.	Santarpia et al., 2014 Italy	case-control	ND	assessment of relationship between gut microbiota and abnormal body weight	4	18/16	18/16	22±4/38±14	3/6	16.67/37.5
5.	Caroll et al., 2018 USA	ND	inpatient	the link between gut microbiota and the phenotype of AN and mood	7	16 (10)/91	16 (10)/91	ND	ND	ND
6.	Million et al., 2018 France	case-control	ND	the link between microbiome of the gut and different types of malnutrition	8	3/30	3/30	ND	ND	ND

ND – not determined

Microbiota and intestinal barrier-related parameters in AN

Two studies included males in the analysis: the first one involved 22 AN patients and 33 healthy individuals, with one male participant in the HC group (4.5%) [12], while the second study involved 18 AN and 16 HC individuals, with three male participants in the AN group (16.67%) and six in the HC group (37.5%) [15]. Another study examined 10 AN patients and 10 healthy persons; however, the stool analysis was performed only in four participants from each group [11]. One of the studies compared the microbiota between patients with restrictive (n = 14) and binge-eating/purging (n = 11) types of AN. In total, there were 25 patients with AN and 21 individuals from the HC group [12]. Another study, including 16 AN patients and 91 healthy individuals, assessed changes in the gut microbiota before (n = 16) and after (n = 10) renourishment [14].

The intestinal barrier-related markers were reported only in three publications. The study involving 22 patients with AN and 33 healthy individuals included citrulline blood levels as a marker of intestinal barrier integrity [16]. The remaining two studies (with 16 AN patients and 91 HC, and 18 AN and 16 HC) determined SCFAs (involved in epithelial nutrition [17]) content in the stool [14, 15].

One study also analyzed digestive differences in AN [16], while other studies examined: the severity of depressive symptoms [14], and different types of malnutrition (AN n = 3, marasmus n = 17, kwashiorkor n = 20) together with gut redox potential [13]. A study with 18 patients with AN and 16 healthy individuals also examined persons with excessive weight (n = 16) [15].

Table 2. **Microbiota and intestinal barrier-related parameters in AN**

	Study description	Microbiota-related outcomes	Intestinal barrier-related outcomes	Relationships with clinical variables	Method	Conclusions
1.	Hanachi et al., 2019	L: alpha-diversity (Chao1 index but not Shannon index); *Eubacterium*, *Roseburia*, *Anaerostipes and Peptostreptococcaceae*; H: *Turicibacter*, *Anaerotruncus*, *Salmonella* and *Klebsiella*	Citrulline (mmol/L): 35±14	– Inverse correlation between Francis score and an abundance of an unknown genus of *Peptostreptococcaceae* family (r = – 0.581); – Positive correlation between Francis score and *Dialister* (r = 0.392), *Robinsoniella* (r = 0.444) and *Enterococcus* (r = 0.488); – Negative correlation between BMI and *Verrucomicrobiaceae* (r = – 0.307) and *Ruminococcacea* (r = – 0.456); – Positive correlation between BMI and *Clostridiales* (r = 0.340), *Turicibacteraceae* (r = 0.390) and *Eubacteriaceae* (r = 0.407); – Negative correlation between hypertransaminasemia and *Desulfovibrionaceae* (r = – 0.403); – Positive correlation between hypertransaminasemia and *Flavobacteriaceae* (r = 0.365), *Coriobacteriaceae* (r = 0.379) and *Turicibacteraceae* (r = 0.373) – Negative correlation between blood citrulline and *Flavobacteriaceae* (r = – 0.379); – Positive correlation between blood citrulline and *Streptococcaceae* (r = 0.432) and *Lachnospiraceae* (r = 0.444)	16s rRNA NGS (V3 and V4 regions)	Microbiota alterations in AN pts correlates with the severity of FIDs and metabolic malfunctions

table continued on the next page

2.	Hata et al., 2019	Humans L: phylum *Bacteroidetes*, genus *B. fragilis* groups; FMT – Gut microbiota of recipient mice clustered according to the feces of AN (G_{an}) and HC (G_{hc}) but with no significant differences regarding diversity (Chao and Shannon indexes); L: *Bacteroidetes* (G_{an}); H: *Firmicutes*, *Fusobacteria*, *Cyanobacteria* (G_{an})	ND	G_{an} – A significant decrease in body mass gain and lower food intake; – Food efficiency value (body mass gain / food intake) correlation with the relative abundance of *Odoribacter* and *Sutterella*; – Altered behavioral characteristics (increased anxiety) with compulsive behavior correlated to *Bacteroidetes* and *Firmicutes* phyla from which the latter was reduced after probiotic intake – Lowered 5-HT levels in the brainstem	16s rRNA NGS (V3 and V4 regions)	AN-induced gut microbiota dysbiosis is linked to reduced weight gain and mental disorders
3.	Morita et al., 2015	AN total L: total bacteria, obligate anaerobes (*Clostridium coccoides*, *Clostridium leptum* and *Bacteroides fragilis* groups, *Streptococcus*), *Lactobacillus plantarum*; ANR and ANBP L: *Clostridium coccoides*, *Bacteroides fragilis*; ANBP H: *Clostridium difficile*	AN (including ANR) L: levels of acetic and propionic acids	ND	16S/23S rRNA–targeted quantitative RT-PCR technology	Microbiota alterations are typical in AN pts

table continued on the next page

4.	Santarpia et al., 2014	No differences in gut microbiota composition	L: SCFAs	ND	Real-time PCR/ culture-based techniques	SCFAs might be an alternative source of energy in AN pts
5.	Caroll et al., 2018	L: microbial diversity even after refeeding	ND	Levels of anxiety and depression in patients with AN before refeeding were associated with the composition and diversity of the intestinal microbiota	16S rRNA gene sequencing	Microbiota alternations are typical for AN and linked with mood disturbances
6.	Million et al., 2018	L: *Lactobacillus plantarum* compared to lean individuals; *Lactobacillus reuteri* compared to overweight and obese individuals; *Bifidobacterium animalis* compared to overweight; H: *E.coli* compared to all other groups	ND	– BMI positively correlated with *Methanobrevibacter smithii* (r=0.20) and *Lactobacillus reuteri* (r=0.44); – In regression model a tendency of higher *M. Smithii* with lower BMI (coefficient=0.43; 95% CI: 0.90 to 0.05, p=0.08)	16S rRNA (V3-V4) amplification	Redox status and control of oxidative stress could be major determinants of animal and specifically human microbiomes

L – lowered, H – heightened, pts – patients, FIDs – Functional Intestinal Disorders, AN – anorexia nervosa, HC – healthy controls, Gan – germ-free animals transplanted with feces from AN patients, Ghc – germ-free animals transplanted with feces from HC, ANR – restrictive-type anorexia nervosa, ANBP – binge-eating/purging type anorexia nervosa, ND – not determined, NGS – next generation sequencing, SCFAs – short-chain fatty acids

Risk of bias assessment

An analysis of the overall risk of bias (ROB) was limited due to restricted information included in the reviewed manuscripts. None of the publications indicated the study design in the abstract and/or title. Two studies did not provide enough information on the criteria of participant selection [13, 14]. A total of two articles did not lay out the criteria for diagnosing AN [14, 15]. None of the studies explained how the sample size was determined. Two studies poorly described the applied statistical methods [14, 15]. Only two papers listed the limitations of the performed experiment [12, 16]. The reporting quality of the included studies was low (n = 3) [13-15] or moderate (n = 2) [11, 12]. Only one study was of high quality according to the STROBE assessment [10].

Mirobiota evaluation

The analytic methods used to determine microbiota composition varied among the included studies. Stool microbiota, according to 16S rRNA sequencing was assessed in four studies [11, 13, 14, 16], and one study detected bacteria according to the real-time polymerase chain reaction method without any information on sequencing [15]. Another study used 16S or 23S rRNA–targeted RT–qPCR technology [12]. Three of the studies used V3-V4 regions for analyses [11, 13, 16] (in one of them PCR was specific for *M. smithii*). The use of various methods precluded comparability among the studies and made conducting a meta-analysis impossible.

Taxonomic analysis of gut microbiota

Microbiota in stool samples was evaluated in all of the reviewed studies. One study determined the composition of gnotobiotic mice fecal microbiota reconstituted with samples from patients with AN [11], while the rest analyzed human samples. The abundance of bacteria phyla was determined in five studies. Three of them used the 16S rRNA sequencing method, one employed 16S/23S rRNA–targeted RT–quantitative PCR technology [12], and one applied real-time PCR/culture-based techniques [15].

The information regarding bacteria diversity was included in four studies (one of them included an animal model) [11, 12, 14, 16]. The differences in microbiota communities were assessed by principal component analysis (PCA) in one study [12]. Hanachi et al. [16] calculated Chao and Shannon indexes to determine alpha diversity. The animal study examined both alpha diversity and beta-metrics (Shannon index, Chao 1, UniFrac or three-dimensional PCA) [11]. The last study which assessed diversity of the gut ecosystem did not provide information on the method used [14]. Four studies in humans found differences in microbiota diversity between the AN and the HC groups [12-14, 16]. Hata et al. [11] did not find any differences in microbiota diversity between mice with microbiota transplanted from the AN patients and from the HC individuals. Caroll et al. [14] found that patients with anorexia had a different diversity of the microbiota compared to healthy individuals, regardless of renourishment.

Only Santarpia et al. [15] found no differences in the gut microbiota composition of AN patients compared to the HC group. Hanachi et al. [16] noted lower *Eubacterium, Roseburia, Anaerostipes*, and *Peptostreptococcaceae* and higher *Turicibacter, Anaerotruncus, Salmonella*, and *Klebsiella* counts in patients suffering from AN compared to healthy individuals. In the Hata et al. [11] study, both AN patients and mice with feces transplanted from these patients had less abundant *Bacteroides* phylum compared to the HC group or mice with microbiota transplanted from healthy individuals. Patients with AN also had a lower abundance of the genus *B. fragilis* compared to healthy individuals, whereas anorectic mice had lower *Firmicutes, Fusobacteria, Cyanobacteria* levels compared to the HC mice [11]. Morita et al. [12] established that the AN group showed differences in microbiota diversity compared to the healthy persons (lower counts of total bacteria, obligate anaerobes: *Clostridium coccoides, Clostridium leptum*, and *Bacteroides fragilis*, *Streptococcus, Lactobacillus plantarum*), regardless of AN type. However, only binge-eating type AN patients had higher levels of *Clostridium difficile* compared with the HC group [12]. The study conducted by Million and Raoult [13] revealed that AN patients had a higher count of *E. coli* compared to both individuals with proper BMI and persons with excessive body weight. The underweight patients showed different microbiota composition compared to individuals with healthy BMI and those whose BMI was too high: lower counts of *Lactobacillus plantarum* compared to lean individuals, lower *Lactobacillus reuteri* counts compared to overweight and obese subjects, and lower *Bifidobacterium animalis* levels compared to overweight individuals.

Relationships between clinical variables and microbiota composition

Transaminases

The relationship between blood transaminase levels and gut microbiota composition was mentioned in one paper [16]. Hypertransaminasemia, defined as a greater than twofold increase in alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) values, was correlated negatively with *Desulfovibrionaceae* ($r = -0.403$; $p = 0.022$) and positively with *Flavobacteriaceae* ($r = 0.365$; $p = 0.04$), *Coriobacteriaceae* ($r = 0.379$; $p = 0.032$) and *Turicibacteraceae* ($r = 0.373$; $p = 0.035$) [16].

Francis score

Francis score (self-administered questionnaire, which quantifies the severity of functional intestinal disorders and its impact on the quality of life) and its association with the microbiota profile was assessed in one study [16]. Patients with functional intestinal disorders had a lower abundance of an unknown genus belonging to the *Peptostreptococcaceae* family ($r = -0.581$; $p = 0.002$) and an increased abundance of *Dialister* ($r = 0.392$; $p = 0.047$), *Robinsoniella* ($r = 0.444$; $p = 0.023$) and *Enterococcus* ($r = 0.488$; $p = 0.011$) [16].

BMI

The relationship between BMI and microbiota composition was described in two papers [13, 16]. Greater malnutrition (expressed by lower BMI) was negatively cor-

related with the *Verrucomicrobiaceae* ($r = – 0.307$; $p = 0.025$) and *Ruminococcacea* ($r = – 0.456$; $p = 0.001$) families and positively correlated with the *Clostridiales* order ($r = 0.340$; $p = 0.013$), *Turicibacteraceae* ($r = 0.390$; $p = 0.004$) and *Eubacteriaceae* ($r = 0.407$; $p = 0.002$) families [16]. In one of the studies, the authors observed that BMI was positively correlated with *Methanobrevibacter smithii* ($r = 0.20$) and *Lactobacillus reuteri* ($r = 0.44$). In a regression model, a tendency of higher *M. smithii* counts with lower BMI was observed ($R = 0.43$; 95% CI: 0.90-0.05; $p = 0.08$) [13].

Food efficiency value

Food efficiency value (interpreted as the ability of a food source to contribute to weight gain) was assessed in one study conducted with the use of gnotobiotic mice. Compared with gHC mice (gnotobiotic mice representing HC microbiome), gAN mice (gnotobiotic mice representing AN microbiome) demonstrated a reduction in food efficiency. The food efficiency value was not significantly associated with any type of bacterial phyla, but it was significantly correlated with the relative abundance of *Odoribacter* and *Sutterella* [16].

Behavior changes

Changes in behavior were described only in the study using an animal model. Analyses were performed in mice at 7 and 10 weeks of age. A generalized linear mixed model revealed no significant differences in motor activity (total distance travelled during 20 minutes) between the gAN and gHC mice. The gAN mice spent significantly more time in the peripheral subsquares of the box at 7 weeks of age, and buried more marbles at 10 weeks of age than did the gHC mice of the same age (indicator of compulsive behavior). Transplantation of microbiota derived from AN patients altered the behavioral characteristics in recipient mice when compared with that of HC-derived microbiota. Compulsive behavior in female gnotobiotic mice at 7 and 10 weeks of age was significantly correlated with the relative abundance of *Bacteroidetes* and *Firmicutes* phyla. The authors also examined whether administration of *B. vulgatus*, which is a predominant species of the *B. fragilis* group in adult humans, could reverse behavioral abnormalities in gAN mice. Both short – and long-term probiotic treatment with *B. vulgatus* reduced compulsive behavior in gAN mice [16].

5-HT levels

5-hydroxytryptamine (5-HT) brain levels were assessed in the study conducted with the use of mice, and the levels of this hormone were lower in the brainstem of gAN mice than in that of gHC mice, implying reduced activity of the serotonergic system. Brainstem 5-HT levels of gAN and gHC mice exhibited a significantly negative association with the number of buried marbles at 7 and 10 weeks of age ($r = 20.377$; $p = 0.023$). There were no significant differences in 5-hydroxyindoleacetic acid (5-HIAA) levels between gAN and gHC mice in any of the examined brain regions [16].

Intestinal barrier-related markers

Intestinal barrier-related markers were described in three papers [12, 15, 16]. In the study by Hanachi et al. [16], subgroups of AN patients with citrulline levels <30 mmol/l presented microbiota profiles close to those of HC groups. There was an inverse relationship between blood citrulline and the abundance of *Flavobacteriaceae* ($r = – 0.379$; $p = 0.036$) and the low abundance of *Streptococcaceae* ($r = 0.432$; $p = 0.015$) and *Lachnospiraceae* ($r = 0.444$; $p = 0.023$) [16].

Propionic acid levels were lower in the AN group (9.3 ± 4.8) than in the HC group (15.2 ± 5.9). Acetic acid levels were also lower in the AN group (30.7 ± 13.2) than in healthy individuals (58.6 ± 27.0); however, this correlation is significant only for restrictive-type AN [12]. Significantly lower fecal SCFAs contents were observed in AN (butyric $p < 0.001$; propionic $p = 0.001$ and acetic acid $p = 0.026$) compared with obese individuals [15].

Discussion

This systematic review aimed to determine changes in the gut microbiota and intestinal barrier markers related to anorexia nervosa. To the best of our knowledge, this is the first review focusing on both microbiota changes and intestinal barrier parameters in this group of patients. In their meta-analysis, Di Lodovico et al. [18] included a greater number of studies concerning the gut microbiome in AN. Some of the papers included by the authors met our exclusion criteria (antibiotic and/or probiotic use before the examination); therefore, they could not be included in this review. It should be highlighted that after antibiotic therapy, the gut microbiota recovers to near-baseline composition after 1.5 months [19]. Inclusion of individuals receiving antibiotics and/or probiotics before the entry into the study could interfere with the results in the analysis. According to Di Lodovico et al. [18], the heterogeneity of clinical and methodological characteristics impedes the generalization of results. The number of studies on patients with eating disorders is scarce, especially those assessing gut permeability. What is more, the number of included participants is inconsiderable and does not allow for a reliable assessment.

In individuals with eating disorders, high variability in weight gain during renourishment is observed [20]. The mechanisms affecting the variation in patients' responses remain unclear. Differences in the gut microbiota community may result in a poor response to nutrition therapy in malnourished patients.

Most of the included studies in our analysis confirm changes in the microbiota composition in patients suffering from eating disorders [12-15]. However, according to the results of the systematic review, a specific, AN-type microbiota pattern does not exist. Numerous factors influence the gut microenvironment. Interpretation difficulties may result from methodological differences concerning sample collection, bacterial DNA extraction, sample population, as well as selection criteria of the control group.

The composition of gut microbiota is closely related to ethnicity and the region of residence, which prevents comparison of results of studies performed on different ethnic groups [21]. Nevertheless, all of the studies included in the analysis showed dissimilarities in microbiota diversity between healthy individuals and anorexia patients. Only in the animal model study, there were no differences between AN and HC microbiota recipient mice, but the animals with microbiota transplanted from the AN patients showed reduced food intake. This observed restriction of food indicates the role of the gut ecosystem in appetite regulation, which was confirmed both in animals and humans [11]. It is worth mentioning that the results of model studies should be treated with caution due to test-controlled conditions and differences between humans and rodents.

In two studies, the count of *Lactobacillus plantarum* was lower in AN patients [12, 13], and the number of *Lactobaccillus reuteri* was correlated with BMI [13]. The model study revealed that *Lactobacillus* abundance is related to higher levels of leptin and lower levels of ghrelin [22]. Morita et al. [12] found a lower count of some *Clostridia* and *Bacteroides fragilis* groups in restrictive and binge-eating types of AN, which was related to lower leptin levels and higher ghrelin levels in the animal model [12]. Patients with AN had lower *Bifidobacterium animalis* counts compared to overweight individuals [13], which was related to lower serum ghrelin and higher leptin levels in the animal model [22].

Carroll et al. [14] proved that patients suffering from AN had different gut microbiota diversity compared to the HC group, including during the period after nutritional treatment. It should be emphasized that nutritional therapy alone is an insufficient treatment for anorexia nervosa [23].

In the studies included in the review, higher levels of bacteria belonging to the *Enterobacteriaceae* family, namely *Salmonella* and *Klebsiella* [16], and *E. coli* [13], were found in AN patients compared to healthy individuals [13]. The *Enterobacteriaceae* family produces caseinolytic peptidase B (ClpB). This protein is a mimetic of anorexigenic alpha-melanocyte-stimulating hormone (alpha-MSH). In rats, *E. coli*-derived ClpB stimulates the secretion of anorexigenic neuropeptide PYY from the intestinal mucosa in a dose-dependent manner [24]. In the animal model study, food restriction-induced immune activation was reflected by changes in the plasma levels of alpha-MSH antibodies IgG and IgM. Alpha-MSH immune complexes lead to the immune activation of melanocortin type 4 receptor whose pharmacological stimulation leads to weight loss and decreased food intake [25].

Gut skewed composition, collectively known as dysbiosis, might be not only the result of malnutrition but also an important factor involved in AN etiology [26]. To explain the role of the gut-brain axis in AN, modern techniques, with an emphasis on metabolomics as the best tool for determining the phenotype of AN, should be applied. From a biological perspective, anorexia nervosa should be considered as an interplay between the gut microbiota, immune activation, and eating behaviors regulated via neuropeptides.

One interesting concept suggests an immune origin of AN where autoantibodies against neuropeptides, neurotransmitters, and hypothalamic neurons regulating food intake are produced resulting in food restriction. The gut mucosa plays a pivotal role in autoimmunity in AN allowing for the transport of microbial metabolites into the peripheral circulation. *E. coli* is able to dysregulate mucin production and adherence of tight junction proteins, and, as a result, impairs integrity of the intestinal barrier [27].

In order to improve clinical management of AN, it is necessary to determine whether the gut dysbiosis observed in patients is the cause, effect, or sustaining factor of food intake dysregulations. It is well known that the gut ecosystem plays an active role in the regulation of the intestinal barrier structure [3, 28]. The interaction between the microbiome and gut mucosa in anorexia nervosa is a promising field of research. Increased intestinal permeability is associated with inflammation and can be provoked by starvation. We found only three studies assessing changes of the gut mucosa in AN patients. Changes in functions of enterocytes and lower SCFAs levels compared to healthy controls suggest impaired intestinal permeability [29, 30].

SCFAs play a pivotal role in gastrointestinal health and immune homeostasis. Changes in SCFAs concentrations are related to depressive and gastrointestinal symptoms, often concomitant with eating disorders [31]. Supplementation of butyrate and propionate, whose lower levels were observed in AN patients [32], improves the integrity of the gut [33].

Colonic hyperpermeabilty may be one of the causes of liver injury [6]. Two studies included in our review confirmed the relationship between changes in hepatic function and the gut microbiota in AN. According to the study by Jésus et al. [34], approximately 30% of AN patients developed hepatic injury as a result of hypertransaminemia [35]. In order to confirm these findings, further studies assessing the interaction between the gut-microbiome-brain and gut-microbiome-liver axis in AN are needed.

Modification of the gut microbiome remains a promising therapeutic goal in the treatment of anorexia nervosa. Change in the composition of the intestinal ecosystem may help to efficiently utilize the nutritional value of food intake, improve intestinal barrier integrity, and regulate appetite. Restoration of the microbiota composition could reduce the severity of inflammation and gastrointestinal symptoms. Gastric problems during recovery may affect the therapy, especially in the initial stage [35]. This form of treatment could be beneficial for reducing depression and/or anxiety symptoms.

The next step in the field of research on the significance of the gut microbiome in AN should be to determine which strains of bacteria have the greatest therapeutic potential in the normalization of weight in these patients. The nutritional therapy should concentrate on the restoration of the intestinal mucosa, and the stimulation and maintenance of eubiosis along with the improvement of nutrition status [3].

To the best of our knowledge, this is the first systematic review confirming changes in both the gut microbiota community and intestinal barrier-related markers in AN patients compared to healthy individuals. The changes in the microbiota composition and intestinal barrier in AN may be considered as the results of self-imposed food

intake restriction and weight loss. There is no clear agreement on the role of the gut microbiota in the pathophysiological processes that cause eating disorders. Nevertheless, the mechanistic investigations confirm the involvement of the gut microbiota in decreasing appetite, loss of weight, and mood symptoms often concomitant with AN.

Limitations

Certain limitations of this systematic review should be pointed out. First, the low number of studies included in the final analysis and the small sample sizes should be taken into consideration. The strict qualification criteria made assessment of many, including the most recent, studies impossible. However, in our opinion, it was necessary to adopt such conservative criteria because a more liberal approach to the evaluation could decrease the reliability of conclusions. Second, the risk of bias assessment indicates low quality of most of the studies. The heterogeneity of methods for microbiota analysis makes it impossible to formulate definite conclusions. A more sophisticated analysis of the gut microbiota could allow for the determination of a greater amount of changes than those obtained in the papers selected for our review. Differences in the examined populations, especially ethnicity and age, may strongly affect the intestinal microbiota community. None of the studies performed a nutritional assessment to determine the dietary habits of the included participants.

Conclusions

1. This systematic review confirms changes in the gut microbiota community in AN patients compared to healthy individuals.
2. A cause-and-effect relationship between changes in the gut microbiome in patients with anorexia and the symptoms of eating disorders remains unclear.
3. No clear consensus as to bacterial taxa that are most relevant to eating disorders has emerged yet.
4. This systematic review confirms changes in the intestinal barrier of AN patients expressed by changes in concentrations of fecal short-chain fatty acids and alterations of enterocytic function.
5. Damage of intestinal barrier integrity and the role of microbiota are poorly documented in patients with AN and require further, well-designed studies.

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