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Intestinal microbiota and Anorexia Nervosa

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SUMMARY

Anorexia nervosa is an eating disorder characterized by a severe malnutrition, an intense fear of gaining weight and a disturbed self-body image. Anorexia is conventionally considered as a multifactorial disease depending on biological, psychological and socio-cultural factors. Among the different biological factors involved in this eating disorder, the gut microbiota has recently gained a lot of attention. Indeed, the identification of an intestinal microbiota dysbiosis in individuals with anorexia has opened new and promising lines of research. These researches focus in particular on the role of intestinal micro-organisms in the functional gastrointestinal disorders associated with Anorexia, in anxiety and depression-related disorders as well as in the regulation of eating behavior. This review aims to present the current knowledge about the putative role of the gut microbiota in the pathogenesis, course and treatment of Anorexia nervosa.

Contents

1. Introduction 12
2. Why gut microbiota may be a critical player during AN ? 12
3. Intestinal microbiota dysbiosis observed in anorectic patients 13

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4. Potential involvement of gut microbiota in anorexia nervosa pathophysiology 15
5. Conclusion 18
Conflict of Interest 18
Acknowledgements 19
Author contributions 19
References 19

1. Introduction

Anorexia Nervosa (AN) is a highly morbid disease characterized by a significant malnutrition (Body Mass Index (BMI) below 18.5 kg m^{-2}), an intense fear of gaining weight, and a disturbed self-body image (DSM-V diagnostic criteria) [1]. This disease mainly occurs in adolescent girls or young women. Prevalence of AN has been estimated to 1.4% for women and 0.2% for men, and steadily increases in most countries [2]. Anorexia Nervosa has the highest mortality rate of any psychiatric illness, with a standardized mortality ratio >5 (ratio of observed deaths in anorectic patients to expected deaths in the general population) [3]. Two subtypes of AN can be distinguished: restricting type (where patients limit their food intake to decrease body weight) and purging type (where patients use self-induced vomiting or take laxatives to counteract food intake) [1]. Anorectic patients often present with comorbid anxiety and depression [4,5]. Treatment of Anorexia Nervosa is currently based on psychological approaches and refeeding approaches. Refeeding is unfortunately reported to be uncomfortable and distressing to patients, and often fail to restore a healthy body fat composition [6]. Weight restoration is however of critical importance to the healing process. Indeed, higher BMI correlates with better outcome after treatment and prevent depression and severe somatic sequalae, including osteoporosis and infertility [7–9]. Anorexia Nervosa is conventionally considered as a multifactorial disease depending on biological, psychological and socio-cultural factors. Unfortunately, the etiology and the pathophysiology of this disease remain incompletely understood and treatments targeting the causal factors of AN are still lacking [10]. As a consequence, current treatment efficacy remains limited since 40% of anorectic patients after 10 years of medical care still show prolonged symptoms and disabilities [11]. This highlights the importance to develop new alternative therapies against AN.

Among the different biological factors potentially involved in this eating disorder, the gut microbiota has recently gained a lot of attention. Indeed, the intestinal microbiota, which corresponds to the community of micro-organisms living in the intestine, has recently been involved in weight regulation, fat storage and energy harvest from diet, as well as in eating behavior, anxiety and depression [12–15]. As these parameters constitute key facets of AN, this has logically raised the question of the potential role of the gut microbiota in Anorexia Nervosa onset and maintenance, which constitutes a new and promising area of research [16–25]. This review aims to present the current knowledge about the putative role of the gut microbiota in the pathogenesis, course and treatment of AN.

2. Why gut microbiota may be a critical player during AN ?

The intestinal microbiota is an ecosystem composed by bacteria, archaea, microeukaryotes (including fungi and protozoans) and viruses, living in symbiotic relationships within the human host. Bacteria outnumber archaea and microeukaryotes in the human microbiome [26]. Recent estimations determined that the number of bacteria in the gut is of the same order as the number of human cells in the whole body (i.*e.* ~4.0×10^{13} bacteria per individual [27]). The dominant bacterial phyla in the gut are Firmicutes (~60–65%), Bacteroidetes (~20–25%), Proteobacteria (~5–10%) and Actinobacteria (~3%) [13]. Despite the consistency of these phyla, the hundreds of bacterial species present in the gut greatly vary between individuals. In contrast to bacterial species variability, the functional gene profiles of bacteria are quite similar between individuals, suggesting the existence of shared core functions [28]. The functions performed by intestinal micro-organisms include digestion and fermentation of nutrients, in particular carbohydrates and amino acids, and production of essential metabolites such as short

chain fatty acids (SCFAs) and vitamin B and K. In addition, the microbiota plays a key role in the development and the stimulation of the immune system, and constitutes an efficient barrier against exogenous pathogens [29].

The abundance and the composition of the gut microbiota is influenced by many factors, including diet [30–32]. Indeed, long-term as well as short-term dietary changes can induce measurable microbial shifts [30,32]. Particular cases such as severe malnutrition were reported to trigger gut microbiota imbalance (also called dysbiosis). Kwashiorkor, for example, is a severe acute form of malnutrition often observed in developing countries characterized by generalized edema, hepatic steatosis, ulcerative rashes and anorexia. This malnutrition was correlated to an altered composition of the gut microbiota, which, in turn, can influence the consequences of this malnutrition [33]. Indeed, transplantation of bacterial species from children with Kwashiorkor into germ-free mice fed with a low caloric density and nutrient-deficient diet resulted in a significantly greater weight loss in recipient mice than those harboring the microbiota of healthy infants [33]. Given that diet strongly influences gut microbiota composition, it is not surprising that many studies have now established that anorectic patients' intestinal microbiota differ in composition from healthy individuals (see below). Indeed, the diet of anorectic patients is strongly altered, both quantitatively and qualitatively (low in fat and calorie intakes, and high in fiber). An obvious interpretation would be that this altered diet reshapes anorectic patients gut microbiota, which may exacerbate or perpetuate AN by modulating weight loss, eating behavior, mood and intestinal physiology. Another interesting hypothesis would be that a gut dysbiosis may precede the onset of AN and triggers the start of a vicious cycle where the aberrant diet of these patients further alters the composition of the intestinal ecosystem, which may then exacerbate the clinical symptoms of this disease (see Fig. 1).

3. Intestinal microbiota dysbiosis observed in anorectic patients

The first results suggesting that the intestinal microbiota from anorectic patients may differ from healthy individuals came from the quantification of specific bacterial and archaeal species, using quantitative PCR, in stools from anorectic patients compared to healthy individuals [34]. These studies observed an increased level in the methanogen *Methanobrevibacter smithii* in the intestinal microbiota from 9 AN patients compared to 20 normal-weight healthy controls [34]. Interestingly, *M. smithii,* as well as other methanogens, improves microbial fermentation and the transformation of nutrients into calories by metabolizing H_2 excess from the gut into methane. This increase in *M. smithii* could thus reflect an adaptive response towards energy extraction optimization from the very low calory diet absorbed by anorectic patients [34]. This increase in *M. smithii* could also be related to constipation, a frequent functional intestinal disorder observed in anorectic patients (see below). Indeed, increase in methane producing-bacteria has been reported in patients suffering from constipation, and more particularly in patients with a constipation-predominant Irritable Bowel Syndrome (C-IBS) [35,36]. In these patients, the load of *M. smithii* is negatively correlated with the stool frequency and there is now some evidence suggesting that methane slows gastrointestinal motility down and thus may contribute to constipation [35–37].

Following this first evidence of gut dysbiosis occurring in anorectic patients, a study confirmed the increased levels of *M. smithii* in fecal samples from individuals with BMI<25 kg m^{-2} compared to individuals with BMI>25 kg m^{-2} [38]. Another analysis, using culture-based experiments, identified 11 previously undescribed bacterial species in a stool sample from a single AN patient [39]. Whether these species are uniquely associated with AN was not addressed. These three first studies were rapidly completed by several large-scale analysis of the microbiota composition in AN patients, that provided new insights in the dysbiosis associated with anorexia [40–46] (Fig. 2). Of note, the dysbiosis occurring in AN patients does not affect only Eubacteria and Archaea but most probably all the intestinal ecosystem of these individuals, which includes viruses and eukaryotes. Consistently, a study focusing on the gut microeukaryotes of a patient suffering from AN identified a decrease in fungi diversity and detected four species previously unreported in the human gut [47]. Again, these results need to be generalized to other AN patients and completed by large-scale analysis of the diversity of viruses and eukaryotes in these patients.

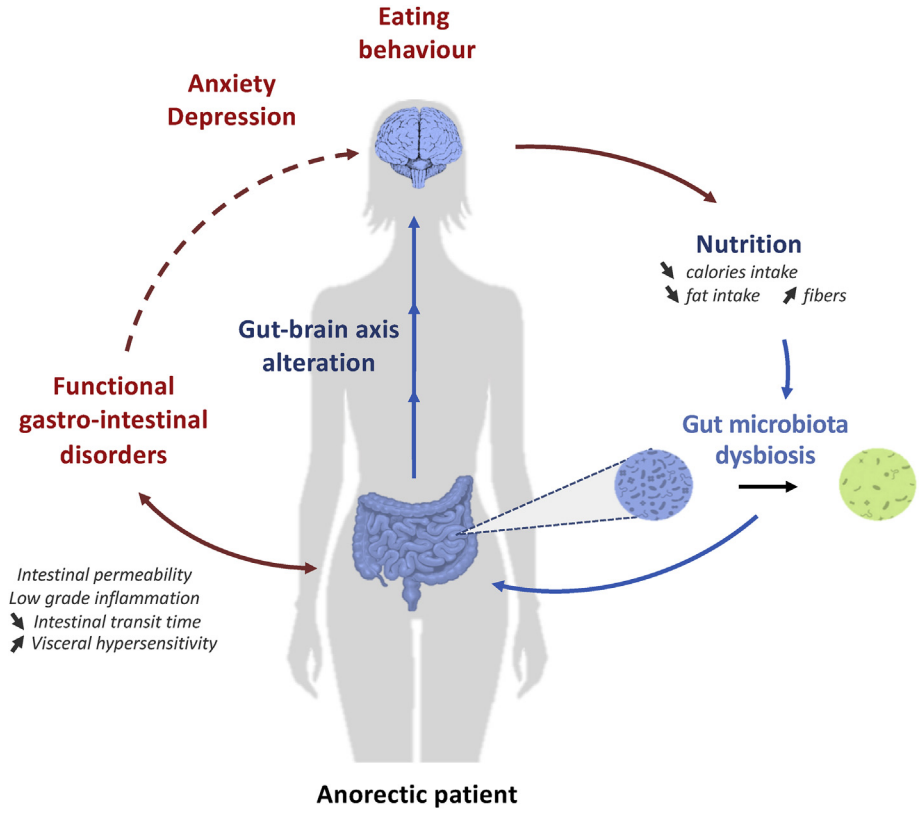


Fig. 1. Putative role of the gut microbiota in the onset and maintenance of Anorexia nervosa. Dramatic changes in diet may profoundly impact the composition of the gut microbiota. The resulting dysbiosis may participate to the onset and/or maintenance of disorders frequently associated with Anorexia nervosa such as functional gastrointestinal disorders, anxiety- and depression-related disorders, as well as appetite dysregulation.

Measurement of bacterial abundance and alpha diversity (*i.e.* bacterial species richness in one individual) of anorectic patients' microbiota are diverging between studies: some studies observed decreases while other studies showed no alteration of these parameters compared to healthy individuals [40–43,45,46]. It is thus difficult to draw definitive conclusions on the modification of these parameters during AN. Regarding the Firmicutes/Bacteroidetes ratio, or the identity of bacterial taxa showing altered levels in anorectic patients, diverging results are also observed between studies (Fig. 2). No clear pattern was actually consistently observed in anorectic patients in currently published studies. There are several reasons that may explain these discrepancies such as the sample collection procedures, the quantification method of bacterial species in feces samples, the selection method of the control group, the choice of timepoint for investigation, or the level of calorie intake by patients [21]. It is also important to note that the number of patients included in these cohorts is quite low (from 10 to 60 patients) compared to other studies focusing on disease-related alteration of gut microbiota (such as obesity or type 1 diabetes, where several hundreds of patients are included).

Thus, there is now evidence that the gut microbiota composition is altered in anorectic patients. However, the exact features of this dysbiosis remain to be unraveled. This will require large and controlled studies where anorectic patients will be precisely phenotyped, in particular regarding their intestinal transit time (stool consistency) or the occurrence of functional gastrointestinal disorder or comorbidities, such as anxiety and depression (see below). This better characterization of the gut

dysbiosis in anorectic patients would be instrumental to understand the exact role of the intestinal microbiota in the different features of AN.

Of note, most of the studies discussed here focused on bacteria recovered from faeces samples. It should be kept in mind that faeces samples only constitute proxies to characterize the gut microbiome and that the actual ecosystems present in the colon or in the small intestine may significantly diverge. It is also important to consider the importance of longitudinal studies in the case of anorexia. In particular, refeeding protocols during hospitalization of these patients may strongly affect their gut microbiota composition. Indeed, the diet of AN patients after hospitalization is quickly changed to a high-calorie diet, rich in fat and carbohydrates. Although some studies have started to dissect microbiota changes before and after refeeding during anorexia, more systematic longitudinal studies would be informative to identify specific micro-organisms associated with either health recovery or relapse [22,41,42,44].

Finally, in addition to these studies of patients' gut microbiota, analysis of gut dysbiosis can be performed in animal models of AN. Interestingly, several changes in bacterial species levels have been observed in the Activity-based Anorexia (ABA) rat model [48] (Fig. 2). This suggests that animal models may constitute a reliable tool to study microbiota-dependent mechanisms involved in AN.

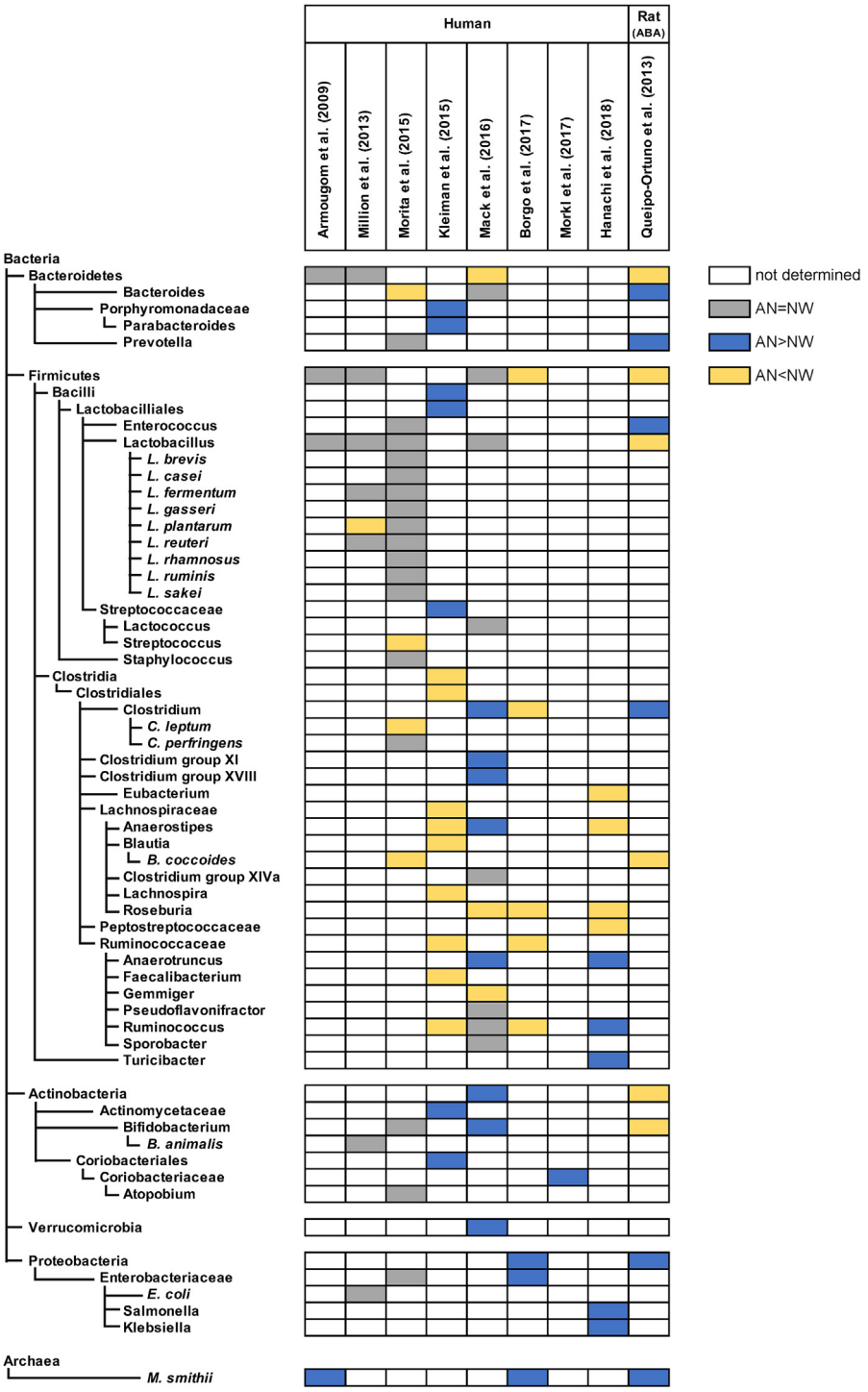
4. Potential involvement of gut microbiota in anorexia nervosa pathophysiology

- Functional gastrointestinal disorders

AN is frequently associated with functional gastrointestinal disorders, which exacerbate patient distress, decrease their quality of life and complicate disease treatment [49,50]. In particular, anorectic patients frequently suffer from delayed gastric emptying, constipation or visceral hypersensitivity. These disorders challenge refeeding strategies resulting in low treatment acceptance and high dropout, increased anxiety linked to food consumption, and finally lead to prolonged nutritional and digestive impairments [49,50].

A link between gut microbiota and gastrointestinal disorders has been proposed in other pathological processes such as Irritable Bowel Syndrome or chronic constipation [51–53]. Indeed, it has been shown that transplantation of fecal microbiota from patients with chronic constipation into recipient mice leads to a reduction of intestinal peristalsis, a decrease in defecation frequency and an increase in gastrointestinal transit time [51]. Similarly, transplantation of fecal microbiota from patients suffering from IBS with diarrhea (IBS-D) into recipient mice is correlated with a faster gastrointestinal transit, dysfunctions of the intestinal barrier as well as anxiety-like behavior [53]. Finally, hypersensitivity to colonic distention of IBS patients can be transferred to rats through their fecal microbiota [54]. Disruption of the intestinal barrier and increased intestinal permeability has been involved in the pathophysiology of functional gastrointestinal disorders [55]. Interestingly, the gut microbiota plays a pivotal role in the regulation of the gut barrier permeability, in particular via the regulation of tight junctions proteins expression [55]. As a consequence, dysbiosis may lead to gene expression dysregulation followed by an increase in intestinal barrier permeability and a low-grade inflammation. Increased intestinal permeability and low-grade inflammation in the colon have been observed in the Activity-Based Anorexia rodent model [56,57]. In AN patients, a decrease in intestinal permeability has been reported in the small intestine, based on urinary recovery of lactulose [58]. Whether intestinal permeability is increased in the colon of anorectic patients remains currently unknown.

According to these established links between intestinal micro-organisms and gastrointestinal dysfunctions, we can hypothesize that the observed gut dysbiosis in anorectic patients may contribute to the onset or maintenance of functional gastrointestinal disorders associated with AN. As already discussed above, the observed increase in *M. smithii* in the intestine of anorectic patients may for instance contribute to constipation through the production of methane by this Archaea, which slows down intestinal motility [36]. The putative role of the microbiota in functional gastrointestinal disorders has recently been highlighted by a study on severely malnourished anorectic patients, which identified correlations between changes in specific bacterial genera with severity of functional gastrointestinal disorders [46]. Characterization of the involvement of gut microbes in these functional


Human
Rat (ABA)
Armougom et al. (2009)
Million et al. (2013)
Morita et al. (2015)
Kleiman et al. (2015)
Mack et al. (2016)
Borgo et al. (2017)
Morkl et al. (2017)
Hanachi et al. (2018)
Queipo-Ortuno et al. (2013)
not determined
AN=NW
AN>NW
AN<NW
Bacteria
Bacteroidetes
Bacteroides
Porphyromonadaceae
Parabacteroides
Prevotella
Firmicutes
Bacilli
Lactobacilliales
Enterococcus
Lactobacillus
L. brevis
L. casei
L. fermentum
L. gasseri
L. plantarum
L. reuteri
L. rhamnosus
L. ruminis
L. sakei
Streptococcaceae
Lactococcus
Streptococcus
Staphylococcus
Clostridia
Clostridiales
Clostridium
C. leptum
C. perfringens
Clostridium group XI
Clostridium group XVIII
Eubacterium
Lachnospiraceae
Anaerostipes
Blautia
B. coccoides
Clostridium group XIVa
Lachnospira
Roseburia
Peptostreptococcaceae
Ruminococcaceae
Anaerotruncus
Faecalibacterium
Gemmiger
Pseudoflavonifractor
Ruminococcus
Sporobacter
Turicibacter
Actinobacteria
Actinomycetaceae
Bifidobacterium
B. animalis
Coriobacteriales
Coriobacteriaceae
Atopobium
Verrucomicrobia
Proteobacteria
Enterobacteriaceae
E. coli
Salmonella
Klebsiella
Archaea
M. smithii

intestinal disorders is essential as it would help to identify new putative therapeutic targets in order to decrease these disorders and improve quality of life and treatment efficiency of AN.

- Anxiety- and depression-related disorders

Psychiatric disorders such as anxiety, depression, affective disorders, substance use disorder or personality disorders are often observed in anorectic patients. For example, up to 80% of individuals with AN will experience major depression at some point in their lifetime, and up to 75% will suffer from anxiety disorders [4,5]. These disorders constitute risk factors for poorer long-term outcomes [4,59,60]. In particular, anorectic patients with a psychiatric comorbidity have higher mortality rates than those without comorbidities [60].

As in the case of functional gastrointestinal disorders, several evidence are now linking gut microbes with psychiatric disorders such as anxiety or depression [14,61]. Fecal content transfer from an innately stress-sensitive mouse strain to non-anxious mice, for example, is sufficient to induce anxiety-like phenotypes in recipient mice [62]. Along the same line, fecal microbiota transfer from depressed patients to rats induce behavioral and psychological features characteristic of depression in recipient rodents [63]. In addition, comparisons of fecal samples from patients with depression with healthy controls highlighted alterations in the levels of several bacterial genera, although no clear consensus has yet emerged from existing human studies [64].

Identifying whether specific intestinal micro-organisms correlate with anxiety and depression in anorectic patients is of interest as it will help understanding the role of the intestinal microbes in these comorbidities of AN. Interestingly, preliminary studies correlating anorectic patients dysbiosis and their level of anxiety and depression identified negative association between the number of observed bacterial species and the level of depression in patients, thereby strengthening the hypothesis of a role of gut microbiota in psychiatric disorders during AN [41].

- Regulation of eating behavior

Appetite in humans is regulated by the balance between energy intake and expenditure. A shift towards low food intake and high energy expenditure normally increases appetite. In the case of AN, patients are unable to adapt their feeding behavior to the body's energy expenditure. This raised the hypothesis that anorectic patients exhibit dysfunctions in appetite regulation and more particularly in the hypothalamic orexigenic/anorexigenic neuropeptides pathways [10,65]. Of course, this dysregulation in neuropeptides controlling appetite may not be the only determinant in this complex diseases and other mechanisms such as abnormally high reward process for starvation and maintenance of thinness have also been proposed for AN [10,66].

Interestingly, recent evidence suggests that appetite may not only be regulated by the body's energy expenditure but also by the energy needs from gut bacteria, which may be distinct from those of the host [15]. Indeed, there might be conflicts between host and microbes in the gut which are fully dependent on their hosts for providing the nutrients necessary for their survival. Thus, intestinal micro-organisms may manipulate host eating behavior to promote their own fitness [67]. Several mechanisms have now been characterized showing how gut microbes manipulate eating behavior of their host [15,67]. These mechanisms rely on the production of a variety of molecules that may either regulate the production of neurohormones involved in mood and eating behavior or act directly as neurohormone-like molecules. For instance, the production of SCFAs, as a result of polysaccharides fermentation by intestinal bacteria, can directly act on enteroendocrine cells of the intestinal epithelium and activate the release of hormones contributing to satiation such as PYY (Peptide tyrosine tyrosine) or GLP1 (Glucagon-Like Peptide 1) [68]. Bacteria may also affect eating behavior by producing

Fig. 2. Alterations in bacteria levels observed in individuals with Anorexia nervosa. List of bacteria with significant increased (blue) or decreased (yellow) levels in patients with AN compared to normal weight individuals (NW) (summary from eight independent studies). Alterations in bacteria levels in a rat model of anorexia (Activity-Based Anorexia model, ABA) are shown in parallel. The phylogeny of bacteria was obtained from the Ribosomal Database Project [76].

compounds reaching the bloodstream and acting at distal sites. The targets of these bacteria-derived molecules are thus not only the local enteric nervous system but many other distant organs including the brain [69].

In contrast to other endocrine organs of the human body, the gut microbiota thus constitutes a "metabolic organ" that has the potential to produce hundreds of different "signaling" molecules. Some of these molecules can be identical to human neurotransmitters or hormones. This is the case for gamma-aminobutyric acid (GABA), produced by strains of *Lactobacillus* and *Bifidobacterium* [70], or for monoamines such as noradrenaline, dopamine or serotonin, produced by diverse strains of intestinal bacteria [69]. The level of these molecules in the body can thus, at least in part, be regulated by gut microbes. Other molecules produced by bacteria can mimic host hormones. This is the case for the bacterial ClpB protein (for Caseinolytic peptidase B protein homologue), that shares some homology with the alpha-melanocyte-stimulating hormone (alpha-MSH), involved in appetite control [15,71]. Whereas intragastric gavage of *Escherichia coli* in mice reduces food intake and body weight, gavage of ClpB-deficient *E. coli* failed to modulate food consumption, suggesting an important role of this protein in host eating behavior [71]. ClpB, which is produced by intestinal bacteria, can be detected in the plasma of both rodents and humans and can directly activate neurons producing pro-opiomelanocortin (POMC), a precursor of the anorexigenic alpha-MSH neuropeptide. ClpB thus constitutes an example of a bacteria-derived molecule linking the gut microbiota with hypothalamic circuits controlling host appetite [72,73].

Taken together, these data on the role of the gut microbiota in the regulation of eating behavior suggest that the observed dysbiosis in anorectic patients may be involved in the disbalance of food intake control and may favor the maintenance or onset of this eating disorder. This hypothesis is strengthened by the recent observation of elevated ClpB levels in a variety of eating disorders [73]. Comparing the levels of bacterial metabolites between anorectic patients and healthy individuals will help to identify new putative bacteria-derived molecules involved in the aberrant eating behavior of individuals with AN.

5. Conclusion

Whereas several studies established that anorectic patients have a dysbiotic intestinal microbiota, the putative consequences of this dysbiosis remain hypothetical. A major challenge in the microbiota field is how to move from observational to mechanistic studies. In other words, how to move from correlations to the demonstration of the role of the microbiota in a given pathology? Much effort is now required to identify how remodeling of the gut microbiota may impact the onset or the course of anorexia. Animal models of AN may constitute interesting tools to tackle these questions [74]. Transplantation of microbiota from anorectic patients to mice and measurement of intestinal physiology alterations, metabolic and behavioral changes specific to the composition of the grafted microbiota would also greatly help to identify enteric micro-organisms that have a detrimental or beneficial impact in AN. This would in particular help to identify bacterial species whose promotion or elimination would improve the efficiency of weight restoration, as well as patients' quality of life. These bacteria may in particular constitute targets to decrease functional gastrointestinal disorders and anxiety or depression disorders associated with AN. The recent publication of preliminary data on the clinical efficacy of fecal microbiota transfer in anorectic patients has confirmed the therapeutic potential of gut microbiota targeting in this disease [75]. Taken together, these experiments will pave the way for the development of innovative treatments, including pharmaconutrition, microbiota modulation or the use of microbiota-derived drugs, as a complement to refeeding and psychological strategies, to fight this frequent and highly morbid disease.

Conflict of Interest

The authors declare that they have no conflicts of interest with the contents of this article.

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Jonathan Breton: Writing-Original draft, Review & editing. **Pierre Déchelotte**: Writing-Original draft, Review & editing. **David Ribet:** Writing-Original draft, Review & editing.

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