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Intestinal microbiota – a key to understanding the pathophysiology of anorexia nervosa?

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Summary

Anorexia nervosa (AN) is a psychiatric disorder related to very serious consequences for physical and mental health of patients. Due to a complex clinical picture, which consists of a number of somatic and mental symptoms, AN remains a serious problem of modern medicine and encourages the search for possible causes of the illness and new, more effective therapies. The recent reports emphasize the role of the intestinal microbiota in regulation of body weight. In this light, the hypothesis that in AN patients there is a significant imbalance of the intestinal microbiota, which contributes to the pathogenesis of the illness, seems interesting. The results of the latest research suggest that abnormal composition of the intestinal microbiota may be an important factor supporting cachexia of AN patients. Detailed analyzes of the composition of the microbiota characteristic for anorexia nervosa could be useful in developing new methods for monitoring and treatment of this illness. This paper aims to present the current state of the art about the role of the intestinal microbiota in the pathogenesis, course and treatment of AN.

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

Introduction

Anorexia nervosa (AN) is a psychiatric disorder related to very serious consequences for physical and mental health of patients [1]. In Poland, AN incidence among girls under 18 years of age ranges from 0.8 to 1.8% [2]. According to Smink et al. [3], AN in the Western countries affects 0.9–4.3% of women and 0.2–0.3% of men and is

considered one of the most dangerous chronic diseases, with mortality rate estimated at about 10% [4].

Malnutrition is considered a major cause of deaths in anorexia, in consequence often leads to anorexia-cachexia syndrome and the associated complications, especially heart failure and electrolyte abnormalities, as well as suicides [5, 1]. AN is characterized by rapidly progressive cachexia, leading to irreversible changes involving complex metabolic and endocrine disorders, and neurological diseases, including reduction in the total volume of the brain [6, 7].

Due to a complex clinical picture, which consists of a number of somatic and mental symptoms, AN remains a serious problem of modern medicine and encourages the search for possible causes of the illness and new, more effective therapies. However, despite the progress of medical knowledge, the etiology of anorexia nervosa remains unclear. Besides the complex psychological mechanisms, an important role of genetic factors in the genesis of the illness is emphasized [8, 9] as well as neuroregulation disorders, mainly in the serotonergic system [10]. Decreased appetite can also be the result of dysfunction of the immune system – with a pathological increase in the concentration of interleukin and interferon [11, 12], and neuroendocrine disorders related to the concentration of neuropeptides in regulating appetite [13]. Unfortunately, the above-described abnormalities do not translate as yet for effective methods of treatment. The role of intestinal microbiota in the pathogenesis and the course of anorexia nervosa has become recently the new and promising area of research.

The paper aim is to present the current state of the art about the role of the intestinal microbiota in the pathogenesis, course and treatment of AN. For this purpose, a review of the literature available in the databases of PubMed and the Google Scholar has been made by typing keywords: *microbiota, microbiome, anorexia nervosa, probiotic, gut-brain axis*, and the time descriptors: 2005–2015.

Intestinal microbiota: definitions, composition, performed functions

The term "microbiota" defines a community of commensal, symbiotic and pathogenic microorganisms residing in the human body [14]. Guts are the habitat for about 10^{13}–10^{14} microorganisms, mainly bacteria *Firmicutes* and *Bacteroidetes*, but also fungi, viruses, and archaea [15]. The gut microbiota significantly affects proper functioning of the human body, and because of its numerous and complex tasks, it is often referred to as the "forgotten organ" [16], responsible, among other things, for the energy balance of the body [17]. It takes part in digestion and fermentation of nutrients and in complex changes associated with the storage of energy obtained from food in the form of short-chain fatty acids [18]. In addition, it is responsible for the production of neurohormones, mainly serotonin [19], polyamines and vitamins B and K as well as the mineral metabolism and efficient functioning of the immune system [20, 21]. Increasingly, the crucial role of the microbiota in the gut-brain axis functioning is also emphasized [22] and even in the modulation of brain development by affecting its function on the basis of complex neuronal, endocrine and immune mechanisms [23].

Intestinal dysbiosis and possible health consequences

The term intestinal dysbiosis describes a condition in which the altered composition of the intestinal microbiota and its malfunction negatively influences the health of the host [24]. Dysbiosis affects the central nervous system by numerous interactions occurring in the gut-brain axis, mainly through an increase in the permeability of the intestinal barrier [25]. By participating in the maturation and exchange of enterocytes, intestinal microbiota facilitates proper functioning of intestinal epithelium, and thus plays a critical role in maintaining the continuity of that barrier [26]. Porous intestinal epithelium is the gateway for proinflammatory bacterial endotoxins such as lipopolysaccharide (LPS) of gram-negative commensal bacteria. LPS directly affects neuronal activity in the limbic system [27], and by the activation of microglia and proinflammatory cytokines, it is responsible for generalized inflammatory response observed in the course of most mental disorders [28].

As the gut microbiota highly influences the development of lymphoid tissue and differentiation of the immune system cells [29, 30], intestinal dysbiosis is responsible for the disorders of normal immune response with the observed advantage of proinflammatory cytokines and, as a consequence, the weakening of broadly understood immunity [31].

The gut dysbiosis also causes a significant decrease in the absorption of essential nutrients, such as vitamins and essential amino acids [32], and thus may lead to maintaining the state of cachexia observed in the undernourished individuals [33].

The role of the intestinal microbiota in malnutrition and cachexia

A close relationship between malnutrition and increased permeability of the intestinal barrier has long been known [34]. Research suggests that disorders of the barrier continuity are closely related to abnormal functioning of the gut-associated lymphoid tissue (GALT) and the onset of the acute phase reaction [34, 35]. In undernourished patients, activation of lymphocytes and enterocytes, a significant increase in plasma concentrations of proinflammatory IL-6 and CRP, were observed – which closely correlated with the severity of dietary restrictions and a decrease in the expression of anti-inflammatory IL-10. Muscle atrophy, especially characteristic of cachexia, is also associated with increased levels of proinflammatory cytokines such as TNF-α, IFNγ, IL-1, and IL-6 [36, 37].

TNF-α and IFNγ seem to be essential modulators of intestinal barrier functioning as they act directly on the tight junctions of intestinal epithelium and thereby increase its permeability [38]. Therefore, it seems reasonable to suspect that malnutrition affects the intestinal barrier just through proinflammatory cytokines [35]. As explained by Genton et al. [35], altered composition of the gut microbiota observed in malnutrition may be both the cause of increased permeability of the intestinal barrier and the local inflammatory process as well as its consequence. These relationships are bidirectional and are intensified mutually as feedback. Bindels and Thissen [39] observed a similar mechanism in cancer cachexia, where inflammation resulting from the proliferative

process impaired the continuity of the intestinal barrier and led to dysbiosis. These authors suggest modifications to the gut microbiota composition using probiotics to seal the intestinal barrier and reduce inflammation in patients with cancer cachexia, which could have a positive impact on the regulation of body mass in this group of patients.

Intestinal microbiota – the role in the regulation of body mass in obesity

There is an increasing number of scientific reports indicating the important role of the intestinal microbiota in the regulation of body mass. Recently, many studies have been addressing the issue of the influence of microbiota on obesity development in both animal [40, 41] and in human models [42–45]. Conclusions from the research clearly indicate the involvement of the gut microbiota in the pathophysiology of obesity and the differences in its composition in overweight and underweight individuals. Turnbaugh et al. [44] in a number of works emphasize the increased numbers of bacteria from groups *Bacteroidetes* and *Firmicutes* in the guts of obese individuals compared with those of normal weight. In obese subjects predominance of intestinal bacteria from the group *Lactobacillus* was also observed [45]. In turn, an interesting study conducted on animals showed that transplantation of the gut microbiota from the obese mice (DIO, i.e., diet-induced obesity) into mice lacking microbiota (GF, i.e., germ-free mice) resulted in the latter in a tendency to accelerate the deposition of body fat than after transplantation of microbiota from lean mice [42]. In another experiment it has been shown that, in contrast to mice with gut microbiota, GF animals do not have a tendency to gain weight despite eating a high-fat and carbohydrate diet [41]. All these reports tend to reflect on the fundamental importance of the gut microbiota in maintaining health and normal body weight and probably that is why the interest, which initiated research into the role of gut bacteria in obesity, quickly covered the opposite pole of disorders, focusing lately on the problem of anorexia.

Intestinal microbiota – the role in the regulation of body mass in anorexia nervosa

Based on the latest reports [46–49], we may suspect that the gut dysbiosis observed in patients with anorexia nervosa significantly affects the course of the illness, and any modifications to the intestinal microbiota can become in the near future a helpful therapy for this disorder.

Animal studies

In 2013, a group of Spanish scientists published the results of the research on rats, which show significant differences in the composition of gut microbiota in the course of anorexia and significant correlation between the composition of microbiota and the plasma concentration of hormones regulating appetite – leptin and ghrelin [50]. In the group of rats with special dietary needs, a significant increase in the number of bacteria and archaea, such as *Proteobacteria, Bacteroides, Clostridium, Enterococcus,*

Prevotella, and *M. smithii,* and a reduction in the number of bacteria *Actinobacteria, Firmicutes, Bacteroidetes, B. coccoides, E. rectale, Lactobacillus,* and *Bifidobacterium,* was noted compared with the group of normally nourished rats. The study also reported a positive correlation between the number of bacteria of the genera *Bifidobacterium* and *Lactobacillus* and plasma leptin levels, and a negative correlation between that same level of plasma leptin and the number of bacteria *Clostridium, Bacteroides* and *Prevotella*. On the other hand, the plasma ghrelin levels correlated positively with the amount of *Bacteroides* and *Prevotella*, and negatively with the *Bifidobacterium, Lactobacillus, B. coccoides,* and *Eubacterium rectale*. These data suggest a significant effect of the gut microbiota on the regulation of appetite.

On the other hand, Jesus et al [51]. in 2014 described the functioning of intestinal barrier in activity-based anorexia in mice. This experimental animal model of anorexia combines increased physical activity often observed in the course of the illness, with limited supply of food. In the experimental mice, there was an increase in intestinal barrier permeability, reduced expression of Claudin-1 and histological changes of the intestinal wall, which may suggest a dysfunction in intestinal barrier in the course of anorexia.

One of the studies in animal models also showed that transplantation of bacteria species *Christensenella minuta* into the intestine of mice affected the inhibition of weight gain in these animals by changing the composition of the gut microbiota [52]. The results of these diverse studies confirm the important role of gut microbiota in regulation of body weight and the development of anorexia.

Human subjects research

In 2009, a group of French researchers examined stool samples from 20 obese patients, 9 patients suffering from anorexia nervosa and 20 healthy volunteers with normal weight in terms of the number of bacteria from groups *Bacteroidetes, Firmicutes* and *Lactobacillus,* and archaea *Methanobrevibacter smithii* [46]. The results confirmed the increased amount of the *Lactobacillus* genus in obese patients. At the same time it was shown that the concentration of archaea *Methanobrevibacter smithii* in patients suffering from AN was much higher than the amount of the species observed in both obese volunteers and those with normal body weight. Apart from a clear increase in the species *Methanobrevibacter smithii* in the microbiota, bacterial profile of patients with AN did not differ significantly from that observed among the group of volunteers and presented a similar number of bacteria of the genera *Bacteroidetes, Firmicutes* and *Lactobacillus*. This growth may result from an adaptive mechanism that allows anorexic patients optimal use of the low-energy food. As the species *Methanobrevibacter smithii* belongs to the methanogens, i.e., anaerobic archaea, in which the main product of respiration is methane produced from the hydrogen and carbon dioxide [53], its increased numbers in the intestine of malnourished person enables efficient transformation of nutrients into calories, and increased energy. Those archaea, by reducing the amount of free hydrogen in the intestine, increase the fermentation yield of non-digestible polysac-

charides (components of fiber) to absorbable, short-chain fatty acids by the bacteria *Bacteroides* and *Firmicutes* [54, 55].

Another explanation for this may be the relationship of methanogens presence in the intestine with the occurrence of constipations, which are more frequent in patients with anorexia [56]. In 1990 Fiedorek et al. [57] observed an increased amount of methanogenic bacteria in people suffering from constipation. Regardless of the causes of these differences, the results of the described research may have important diagnostic and therapeutic effect in the context of eating disorders.

In turn, the study of a single stool sample of patient with anorexia nervosa [58] led to the identification of 11 new species of bacteria of the *Firmicutes* type (n57*), Bacteroidetes* (n52) and *Actinobacteria* (n52). This may suggest the existence of significant differences in the composition of the gut microbiota in the course of anorexia, but on the basis of a single study, it is difficult to assess how the identified species are specific to anorexia nervosa. It is worth noting that the patient participating in the study suffered from extreme malnutrition, and her BMI was 10.4 kg/m^2. Another analysis of stool sample from the same patient revealed the presence of four species of microeukaryotes previously never identified in the human intestine [59]. They were: *Tetratrichomonas sp., Aspergillus ruber, Penicillium solitum* and *Cladosporium bruhnei*. At the same time the diversity of fungi described in the study was limited to 10 species, the presence of which correlated with the type of patient's diet. The results of this study draw our attention to the role of eukaryotes in the composition of the gut microbiota.

Also, in a study of 2015, Morita et al. [48] demonstrated the occurrence of the gut dysbiosis in patients with AN. Comparing the stool samples of 25 women suffering from anorexia nervosa (14 with restrictive type of anorexia and 11 with bulimic type of anorexia) with samples of 21 healthy volunteers from the same age group, Japanese researchers found significant differences in the composition of microbiota of anorexic patients. In these women, regardless of the type of diagnosed anorexia, there was a significant decrease in the intestinal bacteria and the reduced level of bacteria of the genus *Clostridium coccoides, Clostridium leptum* and *Bacteroides fragilis* compared with healthy controls. Patients with anorexia also had less enteric bacteria *Lactobacillus plantarum* and *Streptococcus*. These results confirm the hypothesis that the gut microbiota in patients with anorexia nervosa is significantly different from that observed in healthy subjects.

One of the latest research exploring the relationships between gut microbiota and anorexia is the one carried out in 2015 by Kleiman et al. [49]. The study characterizes gut microbiota composition of 16 patients with anorexia nervosa at baseline and after treatment completion, with weight gain. The results were compared with the results of 12 healthy controls. In AN patients there were observed significant differences in the microbiota composition at the beginning of the treatment and after weight gain. Both the total number of enteric bacteria and their taxonomic diversity were significantly lower in patients prior to treatment. After weight gain, these indicators were clearly improved, but were still significantly lower than in the healthy control group. In addition, there was a significant correlation between the increase in the number and taxonomic diversity of enteric bacteria and the improvement of the mood among

respondents assessed using the Beck Depression Inventory, and the Beck Anxiety Inventory.

It is worth mentioning that in recent years a number of scientific studies have confirmed the effect of gut microbiota on the mood and behavior through its participation in the proper functioning of the gut-brain axis [60–62]. Further it has been demonstrated that modification of the gut microbiota with probiotic bacteria may have a significant effect on reducing the amount and severity of symptoms of depression and anxiety [63–66]. In the light of these reports we may suspect that the changes in the microbiota composition observed in patients with anorexia are closely associated with comorbid symptoms, such as anxiety and depressed mood.

Based on the research of Kleiman et al. [49], it is certainly difficult to draw the unambiguous conclusion that gut dysbiosis is a direct cause or effect of observed anorexia nervosa symptoms. Perhaps it is the result of drastic dietary restrictions used by patient, which does not exclude its participation in the development of comorbid depressive symptoms and anxiety, as well as progressive weight loss. Therefore, it seems reasonable to suppose that the modification of the gut microbiota composition, particularly as regards its diversity, could have important therapeutic effect by reducing the severity of the symptoms of anorexia, normalization of weight loss, and the improvement of the mood of patients.

Further studies require identification of specific species of bacteria, the supplementation or elimination of which would directly affect restoration of normal body weight. The currently ongoing clinical study in Denmark aimed at a thorough analysis of 50 patients with anorexia by the microbiota sequencing, and – for comparison – 50 healthy women, may be helpful [67]. Comparison of the composition of the gut microbiota in such a large group of respondents can provide many valuable data, crucial to the search for new ways of AN therapy. Completion of research is planned for October 2016.

Another study that indicates an interest in the described subject is currently underway in Croatia and is intended to demonstrate the effectiveness of supplementation with the probiotic *Lactobacillus reuteri* in the treatment of gastrointestinal motility disorders in children and adolescents with anorexia [68].

Finally, also the research analyzing the relationship between the development of anorexia and bacterial antigens influencing the regulation of appetite by molecular mimicry, is worth mentioning. Fetissov et al., described the presence of autoantibodies against neuropeptides regulating appetite in patients with eating disorders [69, 70] and related the production of these autoantibodies with changes in the composition of the gut microbiota [71]. Whereas, in 2014 a group of French scientists identified a bacterial protein, ClpB, which can significantly affect the regulation of appetite and the development of eating disorders [47]. This protein is secreted mainly by commensal bacteria of the species *Escherichia coli* and by some pathogenic strains, it induces in the organism the production of autoantibodies of the IgG group that can mimic α-melanocortin and react with its receptor. Hypothalamic melanocortin (α-MSH) is involved in the control of normal food intake and indirectly corresponds to a feeling of satiety. Therefore, the activation of the melanocortin receptor (Mc4r), located in the hypothalamus and other brain regions by autoantibodies against ClpB may induce anorexia by affecting

the regulation of food intake. For confirmation of this thesis the researchers applied *E. coli* producing ClpB protein or bacteria lacking the substance to the gut of mice. In the first group of mice, the decrease in food intake and an increase in the level of anti-ClpB were observed, while in the second group – the changed eating habits or antibody concentrations were not reported.

Further, it was demonstrated that plasma levels of antibodies against ClpB and α-MSH were significantly higher in studied patients with eating disorders such as anorexia, bulimia and BED than in the general population. These data may suggest the importance of ClpB protein expression by intestinal bacteria for the development of eating disorders, however, more research is needed to confirm this theory and its possible use for therapeutic purposes.

Recapitulation

The presented studies are the proof of the important role of gut microbiota in the pathogenesis and course of anorexia nervosa. At the present stage, our knowledge of the complex relationships between the microbiota composition and the development of anorexia is still limited and does not translate into concrete, confirmed by tests, therapeutic effect suggestions. The development of new therapies based on these very promising reports is currently a great scientific challenge. The relationship between gut microbiota and eating disorders undoubtedly requires further multidirectional research, however, our present knowledge is sufficient to assume that microbiota will be an effective therapeutic target in the treatment of anorexia. As the data confirm the beneficial effect of probiotics and prebiotics supplementation on the course of treatment of patients with malnutrition of variable etiology [34], principally through their capacity to modify the composition of the gut microbiota, it seems reasonable to use the same means in the treatment of anorexia. Of course it is difficult to expect that changes in microbiota will become a recipe for all the symptoms of anorexia, but it certainly can help to normalize body weight and regulate it at a later stage of treatment, improve mood and reduce anxiety levels, and thus will make the treatment more effective and less onerous for patients.

The results of previous studies are very promising and create a solid base for exploration of innovative methods of treatment of anorexia nervosa based on the modification of the gut microbiota.

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