Fecal Microbiota Transplantation in Treatment of Anorexia Nervosa

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Abstract

Anorexia Nervosa (AN) is a mental illness marked by a very low body weight and extreme dieting of patients. The severity of this disease is manifested in its high mortality rates. The current treatment for AN includes a restoration of weight and nutrition, and psychotherapy such as family and group therapy or behavioural therapy. Because of the high relapse and drop-out rates for AN treatment, new insights are needed. Physiological complications are an explanation for the high mortality rates and if treated might be a good addition to AN treatment. A recently shown important medical complication in AN patients are problems in the gastrointestinal (GI) tract. GI complications like celiac disease, irritable bowel syndrome and inflammatory bowel disease can make it difficult for AN patients to start eating on top of their mental problems. These GI diseases are associated with a dysbiosis of the intestinal microbiota. Even after weight restoration the gut microbiota is still affected in AN patients and might cause problems in long-term rehabilitation. For treating the microbiota fecal microbial transplantation (FMT) is suggested to be a favorable treatment for restoring a normal gut microbiota. For this reason, there might be a possible positive effect of FMT as an addition to the present treatment for AN. For future research, the positive effect of FMT on the gut-brain axis in AN patients can also be examined because of the importance of a healthy gut on the functioning of the brain.

Introduction

Over the last century the incidence of Anorexia Nervosa (AN) has gradually increased in males and mostly females [1]. Adolescent girls and young women are mainly affected by AN with an average duration of 6 years [2]. AN is a chronic mental illness, an eating disorder, and according to the DSM-5 classified as a combination of low body weight (>15% reduction of the ideal body weight or a body mass index <17,5 kg/m^2), distorted body image and fear of gaining weight [1] [2] [3]. Patients with AN restrict themselves from eating however are paradoxically obsessed with food and eating rituals with symptoms of obsessive-compulsive disorder (OCD) related to symmetry and order [4] [5]. There are different ways of explaining the pathogenesis of AN. For example, the cognitive neuroscience behind these patients. Their rewarding system is reversed, and patients feel rewarded when not eating, as healthy controls normally feel rewarded when eating [6]. In addition, AN patients often show depression and anxiety symptoms. Even after long-term weight restoration these symptoms persist and are possibly related to the pathogenesis and persistence of this illness [7].

Based on these symptoms the best viewed treatment of AN is a two-stage process, consisting of weight restoration and psychotherapy followed by relapse prevention. It is suggested that full-weight restoration results in a better prognosis for full recovery than partial weight gain. The treatment includes family, individual and group therapy, behavioral therapy for the normalization of eating, and nutritional and psychoeducation [8]. Unfortunately, 30-50% of successfully weight-restored patients relapse within a year of discharge and 30-50% of voluntarily admitted patients drop-out prematurely [8]. Thus, establishing additional therapeutic options is of essence for the treatment of AN.

Besides the low recovery rate, the real severity of AN is determined by the high mortality rate, the highest for any psychiatric disorder and an increased risk for suicide [9]. This high mortality rate in AN is either due to suicide or medical complications. Those medical complications are a consequence of the negative impact on most physiological systems, including the reproductive, cardiovascular, and skeletal system [2].

In addition, the gastrointestinal (GI) system is also affected, with an occurrence of >90% of reported GI complaints in AN patients [2]. Recent studies have shown the relevance of the GI tract in the development and recovery of AN [10] [11] [12] [13]. GI complications such as celiac disease, irritable bowel syndrome, inflammatory bowel disease, and gastroparesis can complicate the treatment of AN by making it difficult to eat and by distracting the attention from the goal of weight restoration [1] [3] [14] [15].

Associated with GI disorders is a dysbiosis of the intestinal microbiota. By manipulating this dysregulated microbiota, GI complaints might be repaired, and might thus be a conceivable addition to AN treatment. Manipulation of the microbiome can be done by the usage of different agents like prebiotics (for the encouragement of microbial growth), probiotics (live microbial cultures) and synbiotics (prebiotics and probiotics combined) [16]. Certain probiotics have shown effectiveness in certain lower GI problems, so these agents might be favorable in the treatment of different GI disorders [17]. However, the total favorable effect is still limited. A potential hypothesis is the natural lack of microbial diversity in these substances, which is normally present in the healthy human gut. To help recover this diversity, transplantation of the stool that naturally contains the entire gut microbiome can be used as a treatment for dysbiosis. Fecal microbiota transplantation (FMT) has been shown to be successful in restoring the microbial diversity and is successful in treating different GI infections and disorders like *Clostridium difficile* colitis, inflammatory bowel disease and functional bowel syndromes. FMT might also be favorable for non-GI disorders, however exclusive non-GI disorders with the presence of a decreased microbial diversity [16] [18] [19].

Thus, AN patients have a high prevalence of GI disease symptoms and a high risk of developing GI disorders like irritable bowel syndrome, inflammatory bowel disease, and colitis. FMT is shown to be successful in treating microbial dysbiosis inherent to those GI disorders. Because of this effectiveness, FMT might be a beneficial addition in the treatment of AN, along with the existing two-stage process with psychotherapy and weight gain.

Anorexia Nervosa

Anorexia Nervosa (AN) is a severe mental illness that is defined as an eating disorder were the patient restricts itself from eating. Voluntary self-starvation is not an illness that is recently developed, but it has been reported throughout history and has an increased incidence over the last century [1] [20]. Patients with AN limit their food intake for the goal of extreme weight loss and paradoxically are obsessively preoccupied with food and eating rituals [4]. Patients suffer from a distorted body image and often do not recognize their own sickness. This can lead to an involuntarily participation of treatment and thus might lead to premature drop-outs or relapses [21].

An extremely low BMI as a consequence of starvation or binge-purge eating is a visible symptom of AN. However, behind these obvious symptoms other non-visible features are shown like depression and anxiety [7] [22]. The severity of depression and anxiety is determined by the state of the illness. Thus, malnutrition intensifies the severity of the symptoms and moderate symptoms of depression and anxiety can even persist after long-term weight restoration. The persistence of these behaviours can be an explanation of the pathogenesis and continuance of AN even after full weight-restoration [7] [23].

One way the pathogenesis of AN is explained is through cognitive neuroscience. Patients with AN show a lot of cognitive abnormalities, of which most discussed: the relationship between aberrant reward and hunger. For example, neural networks that are normally activated when hungry show no activation in patients with AN [6]. Also, patients with AN are able to override the reward of food. So, their processing of reward is altered, they delay or avoid the feeling of reward that comes with eating food [24]. Another study concluded that patients with AN feel rewarded when they restrict themselves from eating, instead of healthy controls who feel rewarded when eating. These patients feel some sort of power and achievement when they self-starve, and even patients that are fully recovered from AN are still equally disgusted when they look at normal foods as at rotten foods [25]. Moreover, patients with AN are attentional biased to thin bodies, body shape and eating stimuli compared to healthy controls. In addition, threat-related stimuli elicit less reaction in AN patients. These patients also differ in the processing of perception and the understanding of self and other people [24].

AN is a severe mental illness which is demonstrated by the lethality of this disease. AN has the highest mortality rates of all psychiatric conditions. These high mortality rates are due to medical complications inherent with AN [2] [26] and the increased suicide risk [9]. These findings show the importance of a beneficial treatment. The current treatment of AN is comprised of two-phases: the restoration of weight, and psychotherapy, with the highest goal of no relapse [8]. When treating AN some difficulties are found. First, the ego-syntonic nature of AN. This symptom results in refusal of treatment. Patients only seek help on their own terms and when they are treated, the fear of weight gain and the cravings to start their dieting behaviour intensifies and they drop out [8]. Secondly, as discussed earlier, the reward system of AN patients differ from healthy controls. So, when weight is restored, patients are not rewarded and even repelled by the sight of food, and relapse to their old dieting behaviour [8]. These symptoms of AN are partly the reason why the relapse rates of AN treatment are very high, 30-50% [8]. These numbers show the importance of a different approach or addition to the current treatment of AN.

This treatment is focused on the most important part of AN, weight restoration. However, it is superficially focused on a brain-centric approach. Consequently, these relapse and mortality rates demand an expanding of this narrow approach and use novel insights into the physiological mechanisms of development, maintenance, and persistence of AN.

Physiological Complications in AN patients

Other than the cognitive difficulties of AN which complicates treatment, the discussed mortality rates can be attributed to the inherent physiological complications of AN. Every organ in the human body can be negatively affected by AN, of which some organs are permanently influenced even after nutritional recovery and weight restoration [2] [26]. These medical complications should be recognized, and an appropriate treatment should be provided, which might be the key for a complete treatment of AN. The self-starvation that is seen in AN patients induces the catabolism of protein and fat, which leads to the loss of cellular volume and function and can affect the heart, kidneys, muscles and intestines. Sinus bradycardia for example is the most common arrhythmia in AN patients (95%) [26] and the risk of sudden cardiac death is increased in AN patients [27]. AN is also associated with endocrine dysregulation, which can lead to a reduction in bone mineral density, sex hormones and growth hormone. In addition, there is a high prevalence of fertile preservation in AN patients, which often is dissolved after weight restoration [2] [26]. Furthermore, because of the food restriction, the intestines of AN patients are also affected. Gastrointestinal (GI) complaints are reported in more than 90% of patients with AN [2]. These GI complaints can complicate the weight restoration because of their unpleasant symptoms, and when treated might facilitate an easier treatment for AN.

Patients with AN indicate GI complaints involving swallowing difficulties, coughing during meals, delayed gastric emptying (gastroparesis), early satiety, acid reflux, diarrhea, excessive gas, abdominal pain, food sensitivities and constipation [2] [15]. Those GI complaints characterize GI comorbidities such as celiac disease, irritable bowel syndrome, and inflammatory bowel diseases like Crohn's disease and ulcerative colitis [11] [28].

Celiac disease (CD) is an autoimmune inflammatory reaction in the small bowel triggered by ingestion of gluten. Symptoms and signs of CD vary and can include diarrhea, abdominal pain, and weight loss [15]. Recently the interest of CD in eating disorders is increased and it was shown that there is a significant increase of eating disorders in patients with CD [29]. Another study showed a significant trend towards AN symptoms including traits of perfectionism, body dissatisfaction and other symptoms indicating a possible eating disorder [30].

Secondly, irritable bowel syndrome (IBS) or functional bowel disorder is a functional gastrointestinal disorder (FGID) and characterized by a constellation of non-specific abdominal complaints and overall normal blood levels, which makes it hard to determine IBS using blood examinations [15]. IBS patients reported a lower calorie intake and more disordered eating than healthy controls and was significantly associated with underweight in patients [11] [31].

AN is also associated with inflammatory bowel disease (IBD), which can be divided into two pathologies: Crohn's disease and ulcerative colitis [32]. Crohn's disease can affect the whole GI tract (from mouth to the perianal area) and is characterized by episodic inflammation. Ulcerative colitis is also characterized by episodes of inflammation, but these inflammations are localized in the mucus layer of the colon and can broaden to the rectum. Both Crohn's disease and ulcerative colitis are characterized by diarrhea, loss of appetite, abdominal pain, nausea, and cramping. Patients with IBD and comorbid AN have pro-inflammatory cytokines. These cytokines may lead to downregulating leptin in mice and can change the satiety/hunger mechanisms which lead to early satiety. As a result, the food intake becomes less and can lead to worsening of AN [15]. A delayed diagnosis of inflammatory bowel disease in AN patients might present a barrier for treatment. Patients might be presented with a properly managed psychological treatment but are not treated for their comorbid medical conditions and can thus delay complete recovery [11] [15].

GI complaints worsen through the progression of AN. AN patients that have a prolonged sickness of >5 years have a higher prevalence of GI complaints and AN patients that have a body mass index from <18 kg/m^2 show a 100% occurrence of GI complaints [1].

The treatment of AN patients is mostly focused on weight restoration, for which food intake is crucial. As discussed earlier and explained in *Box 1*, GI complaints can decrease this food intake because of the limiting symptoms. To make it less difficult for AN patients to consume the amount of

food needed for their treatment, an appropriate therapy for these GI complaints might remedy this, and AN patients might experience painless eating and the barrier for increasing the food intake for treatment will become less.

Box 1: Gastrointestinal Disorders – Non-AN Patients

Celiac disease (CD), irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) are disorders that result from disruptions in the gastrointestinal (GI) tract. Some symptoms that are associated with these disorders are abdominal pain, constipation, nausea, early satiety, and weight loss to name just a few [44]. For treatment of these GI disorders strict dietary plans can be made. CD patients have to follow a strict, life-long gluten-free diet, whereas IBS and IBD patients have a less structured and strict diet. For treatment of IBS and IBD, patients have to follow a dietary regimen where trigger foods are identified through a dietary regimen of trial and error [45].

CD is a GI disorder especially located in the small bowel. Because of the uncomfortable abdominal symptoms of CD the food intake of patients might decrease, leading to calorie restriction which can cause disordered eating. Calorie restriction can also be triggered because of a possible gluten-free diet which can lead to increased food awareness and chronic dietary restraint [43].

Additionally, another GI disease is IBS. Patients with IBS show a decrease in caloric intake and show symptoms of underweight in comparison to healthy controls [11]. As well as IBS patients, patients with IBD can show symptoms of a decreased food intake because of early satiety [15].

Dietary-controlled GI disorders will often cause patients to experience uncomfortable and distressing symptoms when consuming food. This may result in creating a conditioned food aversion and patients report a fear of being contaminated during food intake because of unknown trigger foods. Dietary restraint, GI symptoms, food awareness and the fear of consummation of foods can cause the patients to restrict their food intake and develop disordered eating (DE). Disordered eating is used as a term for food restriction and skipping meals [44]. In a study done in 18 Canadian hospitals and a number of 1015 patients, 30,4% of those patients had GI disease of which 45% were malnourished [46]. Malnutrition is recognized as weight loss, loss of body fat and reduced plasma proteins [47]. Patients with IBD also have a high risk for malnutrition, and this in turn can lead to a reduction of food intake [48].

In conclusion, patients that suffer from GI disorders reduce their food intake possibly because of their strict diet, the distressing symptoms after consumption and the non-specific burden of the chronic illness. In patients with CD, IBS and IBD these can act as triggers for the development of DE patterns.

Box 2: Gut Microbiota in Gastrointestinal Disorders – Non-AN Patients

GI disorders like CD, IBS and IBD are associated with changes in the composition or balance of the intestinal microbiota. Dysbiosis of the gut microbiota, a reduction of the microbial diversity and loss of beneficial bacteria in the gut, is closely related to the prevalence of different GI disorders [49]. Every different disorder has a clear microbial signature which is distinctly differentiated from the other diseases and healthy controls. There are alterations in the microbiome that are the same between different disorders. However, there is a so called 'core dysbiosis' in GI diseases and this shows the significant role of the gut microbiome in these disorders [50].

It is shown that dysbiosis is linked with an inflammatory milieu in CD patients. CD patients show an increase in potentially pathogenic species, for example *E. coli*, and a reduction of beneficial species, like *Lactobacillus* [53].

IBS is a broadly studied and most common GI disorder and it is recently shown that there are overall significant differences in the intestinal microbiota between IBS patients and healthy controls [50] [51] [52]. Mainly the microbial diversity is reduced in comparison to healthy controls. Healthy microbiota is characterized by its diversity and richness, which is important for the maintenance of the homeostasis and function of the gut microbiota [53]. Most IBS patients have an increase in *Firmicutes* levels and decreased *Bacteroidetes* levels (table 1). However, there are some inconsistencies in the reported data, because within the *Firmicutes* family there is an overall increase of potentially pathogenic bacteria and a decrease of beneficial bacteria. Nevertheless, these data still support the hypothesis that the gut microbiota is relevant for the pathology of IBS [50] [51] [52]. In table 1, an overview of the changes in microbiome composition in IBS patients using different quantification methods is shown [49].

TABLE 1 REPORTED CHANGES IN GUT MICROBIOTA IN IBS PATIENTS (ADAPTED FROM REFERENCE [49]).

Quantification method	Change
Culture	↓ Bifidobacteria (part of the phylum Actinobacteria), Lactobaccili (part of the phylum Firmicutes), Anaerobes
	↑ Enterobacteria, Aerobes
PCR-DGGE/qPCR	↓ Anaerobes, Lactobaccilli in IBS-D
	↑ Aerobes
FISH	↓ Bifidobacteria
	↑ Firmicutes (phylum that includes Lactobacilli)
Microarray	↓ Bacteroidetes (phylum that includes Bacteroides), Bifidobacteria
	↑ Firmicutes
16S-Pyrosequencing	↓ Bacteroidetes, Bifidobacteria, Actinobacteria
	↑ Firmicutes, Proteobacteria

DGGE: denaturing gradient gel electrophoresis; FISH: fluorescence in situ hybridization; qPCR: quantitative polymerase chain reaction.

Apart from IBS there are other GI disorders that are linked to a disturbed intestinal microbiota, like IBD. Similar to IBS the richness and diversity of the gut microbiota is significantly reduced in IBD, there is a decrease in *Firmicutes* and *Bacteroidetes* and there are shown increases in *Proteobacteria* and *Actinobacteria* [49]. Among IBD, ulcerative colitis and Crohn's disease show differences from each other, where ulcerative colitis shows a reduced concentration in *Bacteroidetes* and Crohn's disease is characterized by a high presence of *Proteobacteria* [50].

Gut Microbiota in AN patients

Recent studies have shown a significant relation between AN and the gut microbiome imbalances [3] [12] [13] [33]. As discussed in *Box* 1 and *Box* 2, in non-AN patients the intestinal microbiota is involved in GI disorders, and GI disorders can be involved in appetite and weight regulation. Hanachi, et al. (2019) showed an overall intestinal microbiota dysbiosis in severely malnourished AN patients. The microbiota in these patients mostly showed an increase of pro-inflammatory bacteria of which some are linked with IBS. This study also showed a strong correlation between gut microbiota dysbiosis and the severity of functional intestinal disorders [12].

A more recent study by Ghenciulescu, Park & Burnet (2021) has shown the involvement of certain commensal species in modulating the appetite of the host and showed the possible contribution of the gut microbiome in the phenotypic core behavior of AN patients. It also summarized the outcomes of different studies that revealed microbial alterations from AN patients in comparison to healthy, normal-weight participants (table 2) [33]. As shown in table 2, most of these outcomes show a decrease in both richness and diversity of the microbiome structure. In microbiome composition an overall decrease of *Firmicutes, Bacteroidetes* and *Lactobacillus* is shown and an increase in *M. smithii*, *E. coli, Proteobacteria* and *Enterobacteriaceae*. The low levels of *Bacteroidetes* are similar to the microbial alterations in GI disorders like IBS and IBD, even as the decrease in beneficial *Firmicutes* and *Lactobacilli* levels (*Box* 2). The rise in *Proteobacteria, E.* coli and Enterobacteriaceae are also seen in GI disorders (*Box* 2, table 1). In addition, high levels of *M. smithii* are correlated with the symptoms of constipation and might interfere with weight restoration in AN patients [34].

So, the gut microbiota is shown to play a role in the pathogenesis of AN. In addition, the dysbiosis of the intestinal microbiota in AN patients is similar to that of different GI disorders in non-AN patients. One of the goals for treating AN is weight restoration which in its turn might also influence the gut microbiota of the patient. Kleiman et al. (2015) showed that the alpha-diversity remained low in samples taken from AN patients at the beginning of hospitalization (T1) and after discharge of the hospital (T2), both compared to samples from a healthy comparison group (HCG). Figure 1 shows the results of the number of observed species between the three groups and the Chao-1 diversity (estimator based on abundance). So, there is an overall low alpha-diversity in T1 and T2 compared to HCG, nevertheless the samples of T1 show a greater difference with HCG than the T2 group. These results suggest that the alpha-diversity of the intestinal microbiota does not significantly increase after weight restoration, but it is trending towards a healthier state [35].

TABLE 2 DIFFERENCES IN GUT MICROBIOME COMPOSITION IN AN PATIENTS (ADAPTED FROM REFERENCE [33]).

Reported differences in AN compared to healthy controls		
Microbiome structure	**Microbiome composition**	**Metabolites**
N/A	↑ *M. smithii* ↔ Firmicutes ↔ Bacteroidetes ↔ *Lactobacillus*	N/A
↔ Abundance (total)	↑ *M. smithii* ↑ *E. coli* ↓ *Lactobacillus reuteri*	N/A
↓Abundance (total)	↓ Obligate anaerobes (*Costridium coccoides* group; *Cl. leptum* subgroup; *Bacteroides fragilis* group) ↓*Streptococcus* ↓ *Lactobacillus plantarum*	↓acetate ↓propionate
↓Richness ↓α Diversity	↑ Bacilli ↑ Coriobacteriales ↑ *Bifidobacteria* ↓ Clostridia ↓ Faecalibacterium ↓ Anaerostipes	N/A
↔ Richness ↔ α diversity ↑ β diversity ↓ α diversity (only in laxative users)	↑ Methanobrevibacter ↑ mucin-degraders (Verrucomicrobia, *Bifidobacteria*) ↑ Clostridium clusters I, XI, XVIII ↓ Bacteroidetes ↓ Actinobacteria ↓ butyrate producers (*Roseburia* spp., *Gemminger* spp.)	↔ SCFAs (total) ↓ butyrate (∼*Roseburia* spp.)
↔ Richness ↔ α diversity ↔ β diversity	↑ Proteobacteria ↑ Enterobacteriaceae ↑ *M. smithii* (if detected) ↓ Firmicutes ↓ *Ruminococcus* ↓ *Clostridium* ↓ *Roseburia*	↓ SCFAs (total) ↓propionate ↓ butyrate ↔ isovalerate, isobutyrate
↓Richness ↓ α diversity	↑Coriobacteriaceae	N/A
N/A	N/A	↓propionate ↓ butyrate ↔ acetate
↓ Richness ↓ α diversity	↑ Enterobacteriaceae ↑ *Klebsiella*, ↑ *Salmonella* ↓ Firmicutes ↓ *Eubacterium* ↓ *Roseburia*	N/A
N/A	↓Bacteroidetes	N/A

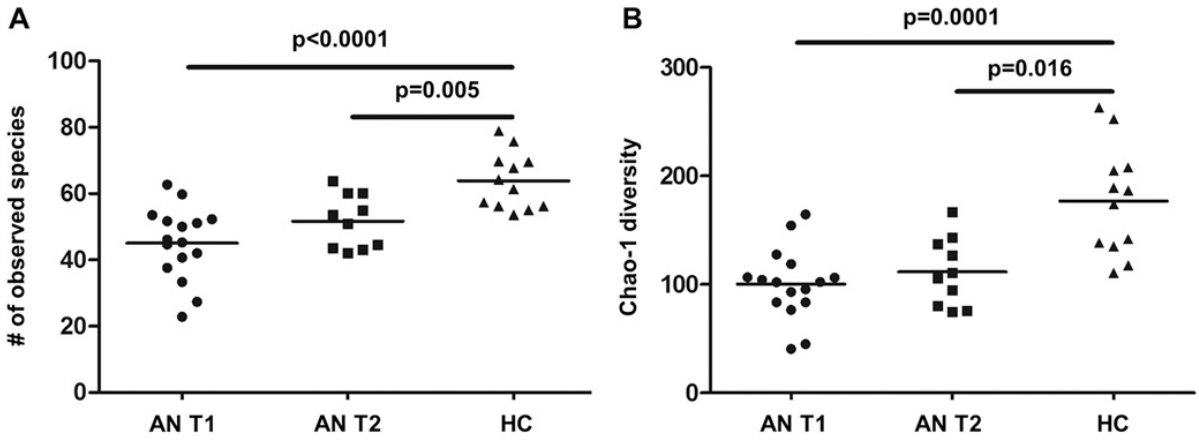

FIGURE 1 THE ALPHA DIVERSITY IN SAMPLES FROM PATIENTS WITH AN AT HOSPITAL ADMISSION (T1; N=16) AND DISCHARGE (T2; N=10) AND A HEALTHY COMPARISON GROUP (HCG); N=12). RICHNESS WAS CHARACTERIZED BY THE (A) NUMBER OF OBSERVED BACTERIAL SPECIES IN EACH SAMPLE; AND (B) CHAO-1 ESTIMATOR OF DIVERSITY. DIFFERENCES WERE COMPARED WITH TWO-TAILED WILCOXON-MANN-WHITNEY TESTS (ADAPTED FROM REFERENCE [35]).

Another study done by Mack et al. (2016) showed the changes in fecal microbiota and severity of GI complaints after weight gain in AN patients. It was shown that the relative abundance of *Bacteroidetes* in specific, was significantly lower in AN patients and decreased further after weight gain. In contrast, the level of *Firmicutes* significantly increased in AN and even more during weight gain. This study concludes that the disturbances found in the gut microbiota of AN patients did not recover after weight restoration. In addition, this treatment was insufficient to thoroughly eliminate the common GI symptoms in those patients [14].

In summary, weight restoration in treatment of AN does not ameliorate intestinal dysbiosis and the often corresponding GI complaints. These persistent complaints might complicate the recovery of AN and can partly be an explanation of the high relapse numbers in weight-restored patients [8]. The treatment of AN patients is focused on weight restoration and the psychopathology, but by adding a treatment of the severely affected gut microbiota the discussed difficulties during rehabilitation might be solved.

Box 3: Treatment of Intestinal Dysbiosis – Non-AN Patients

The healthy gut microbiome is colonized by hundreds of bacterial species and a dysbiosis of this microbiota might influence the health of the host. In human GI disorders it is shown that the intestinal microbiota plays a central role, so modulating this might be considered as a therapeutic strategy to treat diseases like CD, IBS and IBD [54]. Manipulating the microbiome can be done using prebiotics, probiotics, synbiotics and fecal microbiota transplantation (FMT) for example [16] [54].

Prebiotics are nutritional compounds used to encourage beneficial microbial growth and might improve GI health [54]. Two recent studies showed the stimulative effects of prebiotics on specific bacteria in the GI tract and both concluded the small beneficial effect of prebiotics in improving the intestinal microbiota imbalance [55] [56].

Besides prebiotics there are probiotics. Probiotics are live microbial cultures and often used to restore the gut microbiota [16] [54] [56]. Probiotics are, like prebiotics, found to have a small advantageous effect in the treatment of intestinal disorders such as ulcerative colitis [54] and IBS [57]. Probiotics have the ability to colonize the intestinal tract and can effectively use prebiotics to promote their own growth [56]. A combination of pre- and probiotics are known as synbiotics. Synbiotics are shown to have a better ability to promote the persistence of the probiotics than administering these substances individually [56].

However, these materials have shown a generally limited effectiveness in the restoration of the intestinal microbiota. This can be explained by the lack of sufficient microbiological diversity in pre- and probiotics, that is normally represented in the healthy human gut. The ultimate human probiotics are, in a sense, donated feces because they naturally contain the entire gut microbiome. This is a reason why fecal microbiota transplantation (FMT) has a potential of being more beneficial in treating intestinal dysbiosis [16] [18] [19] [54].

FMT is performed by the transferring of fecal microbial ecosystem of a healthy donor into the gut of a recipient to induce therapeutic effects. These stool preparations can be administered in various minimal to non-invasive ways including frozen capsules, nasogastric tubes, or enema [16] [39] [58]. A recent study done by Benech & Sokol (2020) showed the clinical efficacy of FMT in IBS and IBD, but also the importance of a precise characterization of donor profiles and type of disease for therapeutic successes [39]. For treatment of IBS and IBD, the use of FMT has shown to be a promising to significant positive treatment. The results shown after FMT was administered are summarized by an increase in microbial diversity, richness, and a relief or resolution of GI symptoms [16] [18] [19] [39] [58]. In a follow-up in IBS patients, more than 46% of the patients indicated an improved quality of life after FMT was provided [59]. Not only for GI disorders FMT can be a useful treatment, but in different studies it is shown that non-GI disorders, still characterized with a dysfunction of the intestinal microbiota, also benefits from FMT treatment [16] [18].

Fecal Microbial Transplantation in AN patients

As discussed in *Box 3* different therapeutic options can be used for the treatment of intestinal dysbiosis, with FMT as a promising and effective approach for the amelioration of the microbial diversity and relief of GI complaints.

AN patients show a clear dysbiosis of the intestinal microbiota, which is often accompanied by GI disease like symptoms and can make treatment of AN more difficult. A recent study concluded that when GI complaints were treated, AN patients have a better chance at recovery [10]. *Box 3* shows that intestinal microbial dysbiosis in different GI and non-GI disorders is improved and richness and diversity of the microbiota is increased after FMT administration. It is also indicated that there is a relieve or sometimes the GI complaints in GI disorders are resolved. As concluded in *Box 1*, GI

complaints can complicate the nutritional intake in non-AN patients and AN patients, so when resolved, the intake of food can increase again. Therefore, FMT is a reasonable addition to the present two-staged treatment used for AN patients.

Unfortunately, only two recent case-studies about the effect of FMT in AN patients have yet been done. One study showed a steep increase in bacterial diversity (alpha-diversity) as a result of FMT. Also, the richness of the microbiota increased continuously until 6 months and persisted for at least 1 year after the FMT treatment. In this study it was eventually concluded that FMT can be considered as a promising therapeutic strategy for intestinal dysbiosis that results from severe and enduring AN. However, there was no relieve of GI complaints in this patient [36]. This might be explained by the donor selection. The success of FMT is highly dependent on the stool donor, and usually donors are only clinically screened for disease occurrence or pathogens, as in this case study. The most crucial component for the FMT success, microbial diversity, is not analyzed. For a higher success rate of FMT in AN (and more various diseases) an analysis of the microbial diversity should be determining whether the stool donor should be used [37]. This could not only increase the richness and diversity of the intestinal microbiota, but also can relieve or resolve the GI complaints in AN patients.

The other study about FMT in AN talks about the weight gain in patients after treatment. In this case study it is shown for the first time that FMT induced weight gain in a patient with recurrent AN [38]. It is stated that FMT could potentially overcome the microbial dysbiosis and consequent GI complaints that are featured with weight restoration, and thus can facilitate weight restoration [14] [38]. In Figure 2 the effects of FMT on an AN patient is showed in 4 different times of measurement, V0 at baseline, V1 at 6 weeks after FMT, V2 at 12 weeks after FMT, and V3 at 36 weeks after FMT. In Figure 2a it is seen that the bodyweight is increased by 13,8% after 36 weeks compared to baseline, mostly due to the high increase in body fat. This rise is seen despite the reported stable caloric intake. This might be explained because of the increase of certain gut microbes that exceed in extracting calories from food which results in more energy extraction with an unchanged calorie intake. In AN patients the role of gut microbes in host metabolic rates is altered [35], and in this case the resting energy metabolism decreased after FMT, which can contribute to the gain in total bodyweight [38]. Figure 2b shows the decreased levels of *Bacteroidetes* and increased levels of *Firmicutes* in the patient compared to the healthy doner. These adaptations in the gut microbiota are often observed in AN patients as earlier discussed. During FMT treatment there is a high abundance of Verrucomicrobia, especially the *Akkermansia muciniphila* species. This species is associated with improved metabolic health and in both obese as underweight individuals it shows a regression towards a healthy bodyweight. After FMT treatment the *Bacteroidetes* levels increase and the *Firmicutes* levels decrease and the microbial composition is more compatible to the microbiota of the healthy gut donor [38].

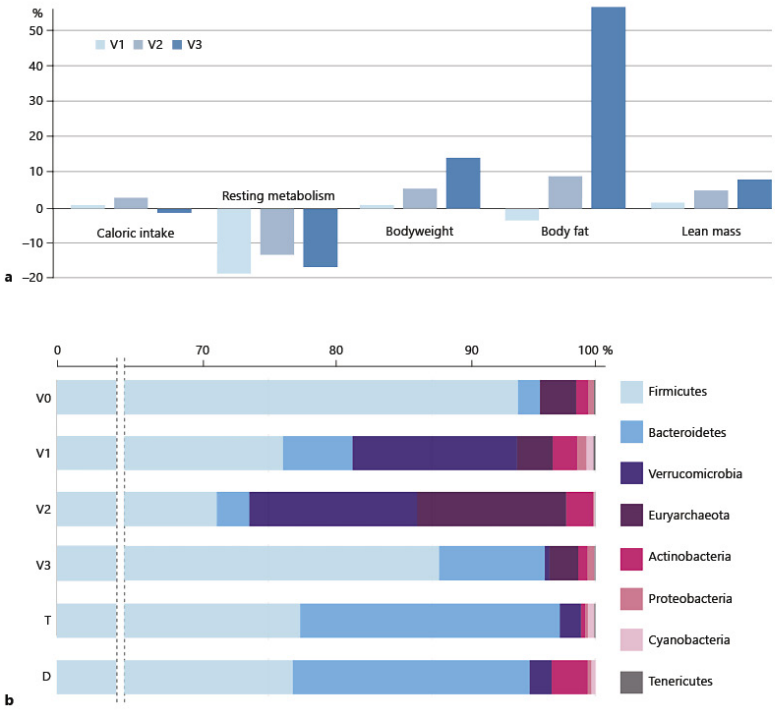

Figure 2 Effect of FMT (A) Percentage change in caloric intake, resting energy metabolism, bodyweight (kg), body fat(kg), and lean mass (kg) at 6 weeks (V1), 12 weeks (V2), and 36 weeks (V3) compared to baseline (V0). (B) Gut microbiota composition with T as composition of microbial transplant, and D as composition of donor gut microbiota (Adapted from reference [38]).

Discussion

Based on the literature presented it can be concluded that FMT is a promising additional therapeutic option for the current multimodality treatment in patients with AN. It is still important to recognize that FMT will be an addition, and that this could not replace the standard two-stage treatment that is currently used for treating AN. For the follow-up of the FMT treatment an inclusion of the administration of pre- and/or probiotics can be beneficial for the maintenance of the transplanted intestinal microbiota. Since pre- and probiotics individually have a lack of microbiological diversity (*Box 3*), their natural supportive function might reinforce the transplanted feces in the host.

Unfortunately, very few research has been done regarding the effect of FMT on AN patients. The general knowledge of FMT in treatment of AN and even GI diseases is modest. Recently, the importance of the stool donor is determined [39] but still very little information is known about the whole procedure and underlying pathways of FMT. This research has gained insight about the possible positive effect of FMT as an addition in the treatment of AN. For future research this effect should be studied in more sizeable patient groups with AN and not only single case studies. It is important that the composition of the gut microbiota of the donor are examined before selecting suitable donors; this might decrease GI complaints. Further research can measure the effect of pre- and/or probiotics after a FMT treatment follow-up in AN patients for a possibly more beneficial and prolonged effect.

Apart from the discussed dysregulation of the microbiota and its direct effect on the GI tract another interesting dysfunction in AN patients that might be affected by FMT is the gut-brain axis. The term gut-brain axis talks about the direct effect of the gut on the brain, and vice versa [40]. A recent study showed that a dysregulation of the gut-brain axis seems to play a key role in AN and discussed new therapeutic treatments for gut-brain modulations and concluded that this can be beneficial for the treatment of AN [41]. The intestinal microbiota communicates with the brain via the axis to influence brain development and behaviour. Stress or depression influences GI illnesses such as IBS and IBD via the gut-brain axis [40]. These GI diseases are associated with a dysbiosis of the gut microbiota. The ability of the intestinal microbiota on the development of the brain and on anxiety and depression in the developed brain is also shown and thus a novel conceptual model of the 'microbiota-gut-brain axis' is proposed [35] [40]. These studies show the importance of a healthy gut microbiota to properly support the brain development or to prevent or treat anxiety and depression. In AN patients, when the microbiota-gut-brain axis is treated, not only the GI complaints might be improved but also symptoms of depression and anxiety that occur in patients with AN can be treated [7].

Furthermore, a recent study talked about the effect of pre- and probiotics on depression and anxiety. This study concluded that probiotics yielded a significant effect for patients with depression and anxiety [42]. Therefore, an addition of pre- and probiotics after FMT treatment might not only maintain the novel intestinal microbiota, but can also have positive effects on the psychological problems in AN.

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