CHAPTER 14

Microbiome and Inflammation in Eating Disorders

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INTRODUCTION

There are roughly as many gut microbial cells we carry as total eukaryotic cells in the human body, each human has a mix of about 500 different microbial species in his or her gut out of a pool of >1000 species. The combined genetic information of these gut microorganisms is about 130 times greater than that of the human genome. Gut microbes constantly interact with dietary factors and host cells. They break down dietary components; influence metabolism, hormone release, and inflammatory processes; and educate our immune system. Recently, compelling evidence emerged that malnutrition and starvation-induced dysfunction of the gut microbiome and its interaction with the brain (“gut-brain axis”) play an important role in the emergence and development of somatic and psychological symptoms in anorexia nervosa (AN) and thus influence the course and outcome of this debilitating disease. Here we review different mechanisms of interaction of the gut microbiome with the host and their relevance for AN and then summarize gut microbiome findings in AN and potential consequences for future research and treatment (Fig. 14.1).

METABOLISM AND BODY WEIGHT

Recent studies have demonstrated the important links between the gut microbiome and the regulation of body weight. This is not surprising, as gut microbes can metabolize many more substrates than human cells, in turn furnishing the host with their often-essential products, fulfilling important symbiotic functions. Certain species thus also allow to extract more energy from the same amount of food than others, effectively linking the gut microbiome to body weight development. For example, bacteroidetes species was correlated with body mass index in over-, normal, and underweight participants. They were decreased in acute patients with AN and normalized during weight rehabilitation of AN patients. The causal role of the gut microbiome in weight regulation is best shown in stool transplantation studies. Mouse models intriguingly showed that the transfer of stool samples from obese to germ-free (GF) mice, bred and born under sterile conditions, leads to obesity. In contrast, the transfer of bacterial species from children with kwashiorkor to GF mice produced significant weight loss and signs of malnutrition. One week of oral antibiotics significantly improved the nutritional status of underfed Malawian children. Transferring stool from bariatric surgery patients to GF mice resulted in significantly less fat mass than colonization with the gut microbiome from obese controls. As AN patients show a significant alteration of the gut microbiome (dysbiosis, see below), an effect on metabolism and weight gain seems more than likely. Interestingly, Mack et al. could show conspicuously different gut microbiome alterations between the restrictive and binge-purging subtype of AN—while patients with restrictive AN are also known to need a markedly increased amount of calories for an equivalent weight gain compared with patients with binge-purging AN.

HORMONES

Although the exact mechanisms are not known, specific changes in hormone levels correlate with the presence of certain gut microbiota. The gut microbiome has been shown to produce hormones, to react to changing hormonal levels of the host and even to regulate host hormone level secretion. Estrogen, e.g., enhances bacterial growth and decreases bacterial virulence in cultured bacteria, while leptin was positively correlated with Bifidobacterium and Lactobacillus in rats. GF
GUT PERMEABILITY AND INFLAMMATION

Increased cortisol as found in acute AN patients in serum, urinary, and salivary samples has been shown to increase intestinal permeability in human studies and animal models alike. In humans, even relatively small stressful situations (public speaking) were sufficient to increase cortisol levels and gut permeability, potentially via activation of mast cells, carrying high-affinity corticotropin-releasing hormone receptors. Thus, in AN, the elevated stress and cortisol levels could contribute to an increased gut permeability. Furthermore, two previous studies revealed a significant intestinal dysbiosis in AN, which was only partially alleviated with weight gain, e.g., lower abundances of Bacteroidetes and carbohydrate utilizing taxa as well as higher abundances of Firmicutes and Verrucomicrobiota. The latter are mucin-degrading and protein-fermenting taxa and are thought to feed on intestinal wall mucins and thus further contribute to increased intestinal wall permeability and even a “leaky gut.” This leaky gut has been shown in an animal model of AN especially in the large intestine and is thought to increase translocation of bacteria, their subcomponents, and bacterial products across the intestinal wall barrier, potentially triggering further immune responses and inflammation. Gut microbe derived—short chain fatty acid and bio-transformed bile acid have, e.g., been shown to act as ligands to specific cell signaling receptors influencing the immune system. Indeed, a recent metaanalysis...
CHAPTER 14 Microbiome and Inflammation in Eating Disorders

showed proinflammatory markers like II-6 and TNF-α to be significantly increased in AN patients,23 evidencing a chronic low-grade inflammatory state. An earlier metaanalysis24 had also shown proinflammatory II-1β to be significantly increased, which remained significant only for restrictive subtype AN in the more comprehensive second analysis. Thus, gut dysbiosis could contribute to increased gut permeability and chronic inflammation in AN.

IMMUNOLOGY AND AUTOANTIBODIES

One of the possible molecular mechanisms linking the gut microbiome with the brain and specifically with the regulation of feeding was demonstrated by the group of Fetissov25, microbiota-induced humoral immune response resulted in antibodies cross-reactive with anorexigenic and orexigenic hormones, such as α-melanocyte-stimulating hormone (α-MSH) and ghrelin, respectively. Indeed, a specific bacterial protein ClpB produced by Enterobacteriaceae was recently identified as an antigen-mimetic of α-MSH, and they found significant correlations between the plasma levels of α-MSH-reactive autoantibodies and psychological traits in patients with eating disorders (EDs), including AN.26 A small study reported increased plasma levels of ClpB in patients with EDs with a strong correlation to α-MSH autoantibodies.27 The mechanism of action of autoantibodies, such as α-MSH-reactive IgG, may involve peptide protection from degradation in circulation preserving its physiological activity, as was shown for ghrelin.28 Also, autoimmune diseases in general are increased in AN as, e.g., shown by a large Finnish population cohort study, with lifetime odds ratios for endocrine autoimmune diseases increased to 2.4, gastrointestinal diseases in general increased to 1.8, and specifically Crohn’s disease increased to 3.9.29 This further links autoantibody production with AN, potentially also instigated by a leaky gut and increased humoral antigen presentation after traversing the intestinal wall. Interestingly, a recent case study of a young patient with comorbid AN and Crohn’s disease showed a significant improvement after anti-TNF-α therapy.30

GUT-BRAIN INTERACTION

The gut microbiome also has important consequences for the brain and its function, starting in early development. This becomes evident, when studying GF mice, e.g., with respect to serotonin metabolism: hippocampal levels of main serotonin metabolites 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT) were significantly increased without contact to a normal gut microbiome during growth.31 Interestingly, 5-HIAA levels were found to be reduced in the CSF of patients with acute AN, while increased levels were found in recovered AN patients.32 The gut microbiome furthermore influences peripheral serotonin secretion by altering number and functioning of enterochromaffin cells in the gut wall, increasing gut peristalsis and reducing transit time, but also entering the bloodstream. Serotonin also plays an important role in mood and anxiety disorders, often comorbid in AN. GF mice also show altered brain-derived neurotrophic factor (BDNF) in the hippocampus, a nerve growth factor influencing neuron growth and protection as well as synapse formation and connectivity, further evidencing the importance of gut microbiota for normal brain development and function. Antibiotics use was shown to reduce hippocampal BDNF and altering anxiety levels.33 Patients with AN also show reduced levels of BDNF in the acute state, which seems to recover upon weight rehabilitation.34 The brain of adolescent AN patients also shows a marked loss in volume of gray and white matter, linked to deficits in neuropsychological functioning and a negative outcome.35 In the animal models of AN, a striking reduction of astrocytes36 and reduced cell neogenesis have been shown by our group. Interestingly, the eradication of the gut microbiome with antibiotics has been also been reported to affect neuropsychological functioning and reduce brain cell neogenesis37 in mice, and supplementation with psychobiotics was able to reverse these changes. However, the extent of the gut microbiome–associated brain changes in AN patients has yet to be analyzed in detail.

GUT MICROBIOME ALTERATIONS IN ANOREXIA NERVOSA

Several studies have so far analyzed the gut microbiome in AN show mixed results.38–41 The groups of Armougom,39 Million,40 Borgo,40 Kleiman,5 Morita41 and Mack42 analyzed cross-sectional samples in 9–55 acutely ill AN patients each, while Kleiman43 and Mack42 also performed longitudinal gut microbiome analysis in 10 and 44 patients, respectively, all using stool sample analyses. Kleiman43 found reduced microbial richness (a measure of α-diversity, the number of different organisms in a sample) in acute and recovered patients with AN compared with controls which were not significant in Mack’s42 and Borgo’s40 samples. However, Mack42 showed a significant increase in richness with weight gain, while Kleiman43 showed a similar trend. Importantly, the number of observed species...
correlated with eating disorder symptom severity and depressive symptoms. β-diversity (a measure of similarity of the microbiome between two different individuals) was increased compared with HC in Mack’s samples showing stronger heterogeneity in AN. Bacterial diversity decreased during weight gain; however, AN patients’ gut microbiome after weight gain still more resembled their own gut microbiome in the acute state than that of HC, evidencing persisting alterations even after weight gain. Increased Firmicutes and decreased Bacteroidetes were observed by Mack and as a trend by Kleiman, low Roseburia species by Mack, Borgo and Armoougom, and Mack also found increased levels of the archaeon Methanobrevibacter smithii. Increased Firmicutes could be partly responsible for the leaky gut mentioned above, due to their mucus-degrading properties. This is further underpinned by an increased amount of branched chain fatty acids shown by Mack and a trend by Morita, which are fermentation products of this protein digestion. They are known to negatively impact appetite by furthering the release of PYY, a gastric peptide, and to also increase depressive symptoms.

**CONSEQUENCES FOR RESEARCH AND THERAPY**

Thus, the gut microbiome might become an essential research and therapeutic target for the treatment of AN and weight rehabilitation to influence food utilization, appetite, and gastrointestinal symptoms as well as neuropsychological functioning and behavior. Moreover, current refeeding practices in AN potentially contribute to a poor outcome. AN patients often maintain a vegetarian or vegan diet low in fat and high in protein and fiber. After admission diet is often quickly changed to a high-caloric diet rich in fat and carbohydrates. Some patients are given oral liquid supplements. Most of these are based on cow’s milk, e.g., an animal-based food product which might increase the growth of inflammation-inducing bacteria. David et al. showed how a purely animal-based diet quickly changed the gut microbiome in healthy participants within days. Thus, we may even induce an iatrogenic worsening of the course of AN by negatively affecting the gut microbiome. These interactions need to be researched. Furthermore, a selective stimulation of the growth of certain bacterial strains by the administration of psychobiotics (“live bacteria, which when applied in adequate amounts, confer mental health benefits”), prebiotics, dietary fibers favoring the growth of these bacteria, and other nutritional interventions are increasingly of interest, as preclinical trials continue to show health benefits. This emerging evidence of preclinical studies encourages the investigation of psychobiotics in mental disorders (e.g., Kelly et al.). A systematic review of 10 RCTs provides initial support for the use of psychobiotics in reducing human anxiety and depression. The authors concluded that there was preliminary evidence for the detection of psychological benefits from psychobiotics, although there were methodological limitations (such as using different strains of bacteria).

Selective psychobiotics, prebiotics, and other nutritional interventions might thus become important additions to AN therapy by altering gut microbiome and gut-brain interaction via several therapeutic approaches. Firstly, one could try to induce more energy retrieval aided by gut bacteria from the same amount of food, effectively increasing weight gain without increasing food volume. Secondly, gut permeability and inflammation could be reduced via favoring specific gut microbiome known for their anti-inflammatory properties, e.g., by prebiotics. And finally, psychobiotics could potentially help reduce depressive and anxious symptoms as well as increase cognitive functioning as recently shown by Bagga et al. using Lactobacilli and Bifidobacteria.

**REFERENCES**

Etiology and Pathophysiology


Abstract
Increasing evidence shows an important role of gut microbial communities influencing metabolism and weight regulation as well as immunologic and inflammatory processes and altering the brain and behavior (gut-brain axis). In anorexia nervosa (AN), microbial diversity was altered correlating with depressive and disordered eating symptoms. More protein- and mucin-fermenting taxa found in AN could induce increased gut wall permeability and explain chronic low-grade inflammation. Bacterial antigen–induced antibodies cross-reactive against hunger and satiety hormones were associated with eating disorder symptoms. Microbiome-targeted nutritional interventions including pre- and probiotics could prove valuable additions to future AN therapy, targeting weight gain, inflammation, and psychological symptoms.

Keywords: Anorexia nervosa; Autoantibodies; Gut permeability; Gut-brain interaction; Inflammation; Microbiome; Nutrition; Psychobiotics.