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Eating Disorders and the Intestinal Microbiota: Mechanisms of Energy Homeostasis and Behavioral Influence

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Abstract
Purpose of Review We reviewed and evaluated recently published scientific studies that explored the role of the intestinal microbiota in eating disorders.
Recent Findings Studies have demonstrated that the intestinal microbiota is a contributing factor to both host energy homeostasis and behavior—two traits commonly disrupted in patients with eating disorders. To date, intestinal microbiota research in eating disorders has focused solely on anorexia nervosa (AN). Initial studies have reported an atypical intestinal microbial composition in patients with AN compared to healthy controls. However, the impact of these AN-associated microbial communities on host metabolism and behavior remains unknown.
Summary The intriguing pattern of findings in patients with AN encourages further investigation of the intestinal microbiota in eating disorders. Elucidating the specific role(s) of these microbial communities may yield novel ideas for augmenting current clinical therapies to promote weight gain, decrease gastrointestinal distress, and even reduce psychological symptomatology.

Keywords Eating disorders · Intestinal microbiota · Metabolism · Brain-gut-microbiota axis

This article is part of the Topical Collection on *Eating Disorders*

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Introduction

Eating Disorders

Eating disorders encompass a range of debilitating psychiatric illnesses broadly characterized by extreme weight and appetite dysregulation [1]. Of the three major eating disorders—anorexia nervosa (AN), bulimia nervosa (BN), and binge-eating disorder (BED)—AN is the only eating disorder to date that has been investigated in relation to the intestinal microbiota [2–4•, 5, 6]. AN is specifically characterized by extreme weight loss or failure to gain expected weight accompanied by fear of weight gain. The disorder typically—but not exclusively—presents during adolescence and affects 0.9% of females and 0.3% of males in the United States [7, 8]. AN has the highest mortality rate of any psychiatric illness with a standardized mortality ratio of 5.86, and only half of patients experience long-term recovery [9, 10]. Moreover, patients with AN often present with other psychiatric and physiological disturbances including anxiety, depression, and gastrointestinal (GI) distress, further complicating the treatment of this disorder [8, 11].

Treatments for acute AN generally involve a combination of clinical renourishment to promote weight gain and psychotherapy to address disordered eating cognitions and behaviors [12, 13]. The evidence base for psychotherapeutic interventions is weak, especially in adults, and clinical protocols for refeeding vary considerably. Refeeding is often associated

with GI distress including pain, bloating, and constipation as well as abnormal body fat deposition [14, 15]. Weight relapse (the re-loss of weight after refeeding) is common and contributes to recurrent presentations [16].

Like all eating disorders, the etiology of AN remains incompletely understood, but as with other complex traits, AN is influenced by an array of genetic and environmental factors [17–19]. The poor understanding of the underlying biology of eating disorders has hampered the development of optimal evidence-based practices to guide clinicians in their approach. Deeper insight into the biological underpinnings of AN has the potential to significantly improve the standard of care and advance the development of effective pharmaceuticals or other treatments for AN. Although many biological factors merit investigation, the intestinal microbiota has recently emerged as a potential target for treatment during clinical renourishment to ameliorate GI distress and improve treatment outcomes.

This review provides an overview of the roles that the intestinal microbiota plays in eating disorders (Fig. 1). The review first focuses on characterizing the intestinal microbiota and then explores the avenues through which these enteric (i.e., intestinal) communities may contribute to the persistence, recovery, or relapse from eating disorders.

The Intestinal Microbiota

The intestinal microbiota is defined as the community of microorganisms, including bacteria, archaea, fungi, parasites, and viruses, that reside within the human GI tract [20]. It has been estimated that this complex community comprises trillions of microbes, equating to a 1:1 ratio of human-to-bacterial cells, with the greatest density and diversity found in the lower GI tract [21]. The specific collection of microorganisms is unique to each individual and the composition of the intestinal microbiota is influenced by myriad host factors including genetics, diet, health status, age, sex, geographical location, and drug exposure [22–30]. Microbial dysbiosis—an imbalance in the expected prevalence of microbial species in the intestinal niche—is often associated with various diseases [27]. The vast majority and most well researched of these microbes are bacteria, which are the focus of this review. However, the role of fungi and viruses should not be overlooked, as these kingdoms are emerging as relevant to other GI diseases, such as inflammatory bowel diseases (IBD) [31, 32].

Perhaps more impressive than the sheer number of microorganisms are the robust and significant relationships this community has with human health and disease. The intestinal microbiota is pivotal for detoxifying ingested drugs, training the human immune system to distinguish between pathogens and commensal organisms, and synthesizing vitamins including B vitamins and vitamin K [30, 33, 34]. Recently, the gut microbiota has been implicated in substantially influencing host weight regulation and energy harvest from the diet (i.e., extracting calories from food ingested) as well as modulating host behavior via direct and indirect pathways [35, 36]. As a

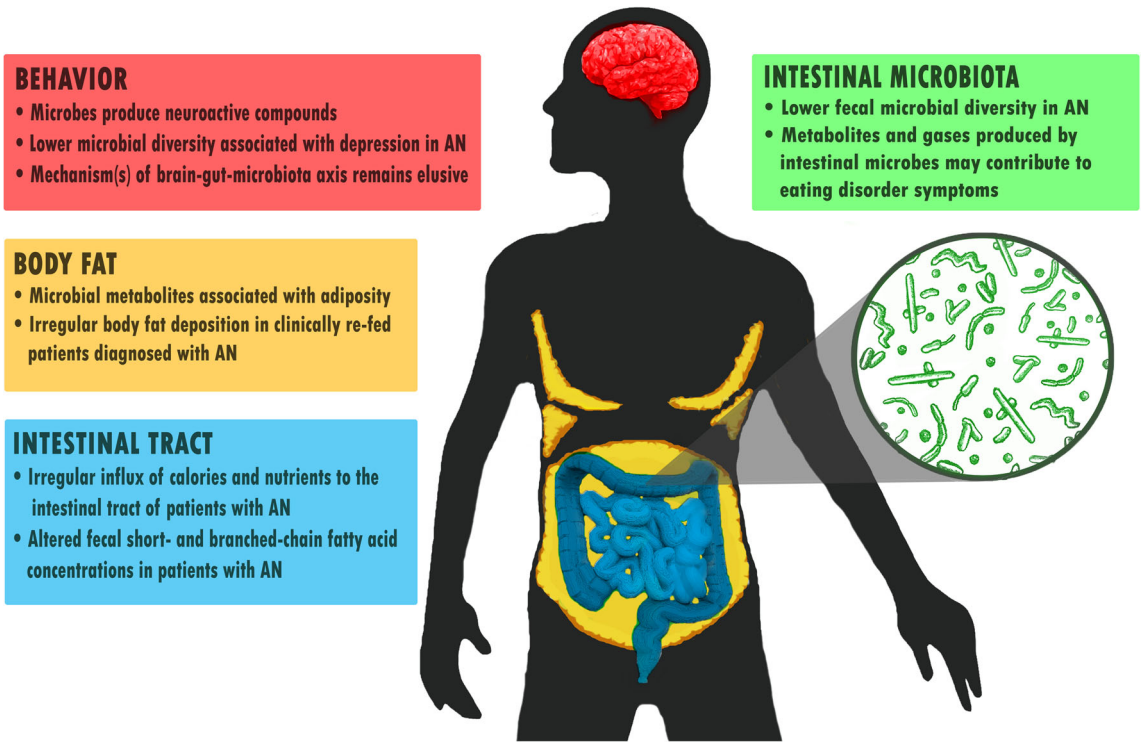

Fig. 1 Microbial influences in anorexia nervosa (AN). Brain and intestine models adapted from originals courtesy of Digimation, Inc.

result of these findings, attention to the intestinal microbiota has increased over the past two decades in metabolic and GI disorders including obesity, malnutrition, IBD, and colorectal cancer [37–40]. There is also nascent interest in the intestinal microbiota's role in Parkinson's disease and neurodevelopmental disorders such as autism [41, 42]. Given that gut microbiotas influence both weight regulation and behavior, two hallmarks of AN, initial investigations into the intestinal microbiotas of patients with AN have yielded intriguing preliminary results.

Energy Homeostasis and the Intestinal Microbiota

Accumulating evidence from both animal studies and, more recently, human clinical trials, supports the notion that the intestinal microbiota plays a substantial role in nutrient extraction and host metabolism. The majority of intestinal microbiota research has focused on mechanisms by which gut microbiotas either directly produce metabolites or indirectly regulate host metabolic pathways to influence host energy homeostasis. It is highly plausible that the metabolic functions of these microbial communities are affected by the dysregulated influx of nutrients and calories to the GI tract in patients with eating disorders.

Evidence for a Role of the Intestinal Microbiota in Energy Homeostasis

Germ-free (GF) rodents—mice and rats born and living without any microorganisms—are a powerful animal model to investigate both the causal role of the intestinal microbiota in human diseases and its direct effect on host physiology and metabolism. Compared with conventionally raised rodents (i.e., rodents living with microorganisms), GF rodents display slower GI transit time and an enlarged cecum (a pouch located between the small and large intestines) caused by accumulation of mucous glycoproteins [43, 44]. GF rodents also have less body fat and consume approximately 30% more daily calories of chow to maintain normal growth compared with conventionally raised rodents [45]. These unique phenotypic characteristics suggest that the intestinal microbiota substantially interacts with its host to promote intestinal transit, digest nutrients, and assimilate energy to influence host metabolism.

Transplantation studies, in which GF mice are colonized with human fecal microbiotas (as a proxy for intestinal microbiotas), permit investigators to observe metabolic, physiological, and behavioral outcomes resulting from the introduced microorganisms. In a seminal study by Ridaura et al., investigators colonized GF mice with fecal microbiotas from either obese or normal-weight human twins [46•]. Over a 2-week colonization period, the GF mice colonized with microbiotas from obese humans developed more adiposity despite no significant difference in food intake, suggesting a greater capacity for the obese-associated intestinal microbiotas to extract calories from the standard chow diet. This basic study design has since been replicated to probe into functions of other microbial communities implicated in a variety of metabolic diseases. In one such recent study, GF mice were colonized with stool provided by women who had undergone either Roux-en-Y gastric bypass or vertical banded gastroplasty 10 years prior or who were obese controls matched to the pre-surgery BMI of the women in the surgical groups [47]. Notably, formerly GF mice colonized with fecal microbiotas from both bariatric surgery patient groups (i.e., Roux-en-Y gastric bypass and vertical banded gastroplasty) displayed less fat mass compared to mice colonized with the obese participants' stool, indicating that the decreased fat deposition was driven by these surgically altered microbial communities. These findings also demonstrate that clinical interventions can indeed effect lasting compositional and functional changes to intestinal microbial communities. Although compelling and highly supportive of the gut microbiota as a major contributor to host metabolism, these human transplantation studies must be interpreted cautiously within the context of a small number of donor samples (i.e., 2–5 human donors per group) and/or the almost exclusive use of male GF mice [38, 46–48]. Replications and extensions using both male and female GF mice and more donor samples will contribute valuable data to this field.

Initial attempts to translate these animal studies into clinical investigations are underway. Fecal microbiota transplantations, by which a liquid preparation of stool from a healthy human donor is introduced following a bowel lavage to the GI tract of a recipient, have been shown to improve insulin sentivity in a group of obese males (n = 9) 6 weeks after treatment [49]. In contrast, a randomized double-blind placebo-controlled trial (RCT) evaluated changes to metabolic parameters in prediabetic obese men (n = 57) after a 7-day course of antibiotics in order to investigate the effects of depletion, rather than augmentation, of the intestinal microbiota. The investigators reported decreased microbial diversity and secondary bile acid concentrations in the vancomycin antibiotic group at 7 days, but saw no changes in insulin sensitivity at either the 7-day or 8-week follow-up compared to the placebo group [50•]. Although no study of antibiotics in AN has been conducted that analyzed the intestinal microbiota, antibiotics such as erythromycin and other prokinetic agents have been used clinically to accelerate gastric transit time and weight gain and reduce GI distress [51, 52]. Repeating such clinical trials and including pre- and post-measures of the intestinal microbiota and other metabolic indices could be a valuable addition to the AN treatment literature and a first step in understanding whether alterations to the intestinal microbiota play a role in recovery and relapse.

Mechanisms

Crosstalk between microbes and host intestinal epithelial cells has emerged as an exciting area of research to explore mechanisms by which specific microbes, and/or the production of specific microbial metabolites, may influence host physiology and metabolism. A currently popular hypothesis proposes that certain microbial communities driven by environmental stressors alter GI physiology to increase host energy assimilation [53]. To investigate this hypothesis, Chevalier et al. colonized GF mice with fecal microbiotas from mice subjected to either room temperature or cold (6 °C) housing conditions [54]. The authors reported that the cold microbiota-colonized mice displayed an increased capacity to absorb calories via greater small intestinal and microvilli length resulting from reduced intestinal epithelial cell apoptosis (programmed cell death). This intestinal epithelial adaptation to increase the total GI absorptive surface is a potential mechanism orchestrated by the intestinal microbiota to improve caloric harvest for fat deposition and mitigation of the cold stressor.

Another area of research investigating the crosstalk between enteric microbes and host intestinal epithelial cells pertains to the metabolites those microbes produce. Enteric microbial-derived metabolites, namely short-chain fatty acids (SCFAs) and secondary bile acids, have also been shown to be significant contributors to host energy homeostasis. SCFAs, specifically acetate, propionate, and butyrate, are derived from bacterial fermentation of complex polysaccharides and supply up to 10% of the host's daily caloric intake [55]. Indeed, butyrate is the primary energy source for colonocytes while acetate and propionate are substrates for hepatic lipogenesis and gluconeogenesis, respectively, to produce lipids and glucose for host utilization [56, 57]. In addition to providing energy, SCFAs can bind to specific distal ileum and colonic G-protein coupled receptors (GPCRs: GPR41 and GPR43) to induce the secretion of gut hormones from intestinal enteroendocrine cells. These hormones, such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), stimulate insulin secretion and inhibit gastric motility, respectively [58, 59]. Secondary bile acids are produced in a two-step process by which bacteria in the distal ileum and colon first deconjugate and then dehydroxylate unabsorbed primary bile acids to create secondary bile acids. Both primary and secondary bile acids aid in lipid digestion and cholesterol metabolism and can also function as signaling molecules to alter glucose homeostasis and brown adipose tissue metabolism [60].

Behavior Modulation and the Intestinal Microbiota

In addition to their role in energy homeostasis, enteric microbes and their metabolites can modulate mood and behavior. The knowledge that the central nervous system (CNS) interacts with our digestive tract (the "brain-gut axis") has existed since the discovery of the enteric nervous system, a collection of 200–600 million neurons that line the GI tract, over a century ago [36]. However, the discovery that intestinal microbes can influence neurological function is much more recent and has come to be known as the "brain-gut-microbiota axis" [36, 61•]. Elucidating the mechanism behind this phenomenon is an active area of research and one that is of particular relevance to eating disorders given their clear relationship with psychological function, eating, and behavior.

Evidence for a Brain-Gut-Microbiota Axis

As with research into the intestinal microbiota's role in energy homeostasis, the use of GF rodents has greatly benefited preclinical studies investigating the brain-gut-microbiota axis. A pioneering study by Sudo et al. demonstrated that there are basal differences in various biomarkers of the hypothalamic-pituitary-adrenal (HPA) axis stress response between GF and microbe-colonized mice, with GF mice experiencing more aggressive stress responses [62]. This exaggerated response in GF mice was reversible when the mice were colonized with microbes at an adolescent age (4 weeks old), but not when they were first colonized with microbes as adults (greater than 6 weeks old). Subsequent studies have demonstrated that compared to mice with "normal" intestinal microbiotas, GF mice exhibit a number of differences in brain and neuron morphology, anxiety-like behavior, and levels of serotonin and brain-derived neurotrophic factor [63–68].

One powerful approach to observe the effect that enteric microbial presence has on disease symptoms is the manipulation of the intestinal microbiotas of mouse models for particular neurological diseases. For example, Sampson et al. recently demonstrated that GF conditions ameliorate the motor deficits displayed by a murine model for Parkinson's disease [41]. Additionally, when those GF mice were colonized with microbiotas from individuals with Parkinson's disease, their motor deficiencies worsened compared with genetically identical GF mice colonized with microbiotas from healthy humans. Similarly, Hsiao et al. reported that targeted treatment of a mouse model for autism spectrum disorder (ASD) with *Bacteroides fragilis* improved both behavioral and gut permeability symptoms [69•]. They also observed that when wild-type mice were given a particular metabolite (4-ethylphenylsulfate) that is typically elevated in the ASD mouse model and modulated by *B. fragilis*, they developed some of the anxiety-like behavioral symptoms characteristic of the ASD mouse.

Another intriguing line of evidence to support the existence of a brain-gut-microbiota axis pertains to prebiotics, which are compounds that support the growth of particular microbes. Recent evidence in mice demonstrates that serial administration of fructooligosaccharides (an artificial

sweetener) and galactooligosaccharides significantly alters bacterial abundances in the intestinal microbiota and decreases both anxiety-like and depressive-like behavior [61•].

These converging lines of preclinical evidence, combined with studies that establish dysbioses in the intestinal microbiotas of patients with certain disorders, have encouraged a number of human clinical trials investigating the therapeutic application of microbes for psychiatric disorders. Many such trials—using so-called "psychobiotics," or living organisms that offer mental health benefits upon ingestion—are currently underway [70]. While the popular media tend to focus on psychobiotic clinical trials that achieve positive results, negative results are also quite common. For example, a recent double-blind, placebo-controlled RCT investigating the efficacy of probiotics in the treatment of depression found no marked difference in outcomes between the placebo and probiotic groups [71]. A meta-analysis of RCTs investigating the efficacy of psychobiotics in treating anxiety and depression revealed that many RCTs report different results, with overall preliminary evidence existing to tentatively support the use of psychobiotics in treating these disorders [72]. Importantly, many of the RCTs employed different strains of bacteria, complicating efforts to pool and summarize the results.

Mechanisms

Hypotheses explaining the mechanisms by which enteric microbes influence mood and behavior abound, and at present, propose many distinct pathways for this complex, multifaceted process. Generally, the hypothesized mechanisms focus on two aspects of the brain-gut-microbiota axis: (1) which compounds (either produced directly by bacteria or whose production bacteria promote) have the ability to influence mood and behavior and (2) how those compounds might interface with other elements of the nervous system.

Enteric bacteria either directly produce or stimulate the production of an expansive list of neuroactive compounds, to such an extent that the intestinal microbiota has been referred to as a "neglected endocrine organ" [73]. The most notable compounds produced or promoted by enteric microbes in both human and murine hosts that may influence mood are neurotransmitters (including dopamine, serotonin, acetylcholine, and γ-aminobutyric acid) and some of their precursors (e.g., tryptophan, kynurenine) [74–78]. Certain bacteria also exhibit increased growth in the presence of catecholamines, suggesting a potential for enteric bacteria to modulate behavior by removing neuroactive compounds [79].

Where these molecules travel after their production in the gut and how they induce a behavioral effect remain active areas of inquiry. One proposed mechanism involves the vagus nerve. Bravo et al. demonstrated that the positive emotional effects of colonization with *Lactobacillus rhamnosus* (JB-1) were negated after vagotomy in mice, suggesting that the vagus nerve (the tenth pair of cranial nerves, involved in controlling the upper digestive tract and other organs of the chest and abdomen) may serve as a conduit in the brain-gut-microbiota axis [80]. It is also uncertain whether any of the metabolites or neuroactive compounds produced by bacteria can cross the blood-brain barrier (BBB) to influence neurological functioning. This remains to be established, though it is possible that they may be able to reach circumventricular organs lacking a BBB. Complicating this hypothesis, it has been shown in mice that the presence of enteric microbes results in a less permeable BBB, compared to the BBB of GF mice [64].

Intestinal Microbial Communities in Eating Disorders

Animal studies have demonstrated that the intestinal microbiota is intimately linked to traits exhibited by individuals with eating disorders, such as dysregulated energy homeostasis and behavior. However, characterization of enteric microbial communities from individuals with eating disorders is a necessary step toward establishing a clinical link between those communities and these illnesses. To date, the literature characterizing the intestinal microbiota in patients with eating disorders has focused on AN.

Evidence for a Role of the Intestinal Microbiota in Patients with Eating Disorders

Initially, the microbial profiles of a small number of patients with AN ($n = 9$) were compared to obese ($n = 20$) and control ($n = 20$) groups [2]. Using polymerase chain reaction (PCR), this study found significantly higher levels of *Methanobrevibacter smithii* (a commensal enteric microbe belonging to the Archaea domain) in patients with AN compared to controls. As *M. smithii* can reduce CO_2 in the presence of H_2 to produce methane, a gas that is associated with delayed intestinal motility, the authors speculated that this microbe may promote constipation, a symptom frequently observed in patients with AN [81]. Given that the intestinal microbiota harbors up to 1150 different bacterial species, and this study only investigated four microbial groups using a relatively narrow approach, a broader characterization was warranted [82]. Using a culturomics approach (large-scale culturing of microorganisms combined with molecular identification of cultured microbial colonies), investigators identified 11 new bacterial species in a stool sample from one individual with AN [3]. However, because the main objective of the study was to develop a novel technology, the researchers only used one stool sample as a template and therefore could not draw any direct association

between the 11 novel bacterial strains and the clinical status of the donor.

Although these studies collectively suggest an altered intestinal microbiota in patients with eating disorders, broad molecular methods provide a more comprehensive and unbiased characterization of these complex communities. Kleiman et al. was the first group to characterize the intestinal microbiota of patients with AN using high-throughput sequencing of the 16S rRNA gene, comparing female patients with AN before (n = 16) and after (n = 10) clinical refeeding at an inpatient specialist unit to healthy controls (n = 12) [4•]. The authors reported lower microbial diversity in patients with AN at both time points compared with controls. Interestingly, higher levels of self-reported depression in patients with AN at hospital admission were significantly associated with lower microbial diversity, suggesting a brain-gut-microbiota interaction in this population.

Another PCR-based investigation (employing reverse transcription quantitative PCR) collected stool samples from patients with restricting type AN (n = 14), binge-eating type AN (n = 11), and controls (n = 21) [5]. Compared with controls, patients with AN had lower abundances of specific taxa belonging to *Streptococcus*, *Clostridium*, and *Bacteroides* genera and lower concentrations of the fecal SCFAs acetate and propionate. Most recently, results from the largest recruited cohort of patients with AN to date replicated the previously reported dysbiotic enteric microbial communitiy in patients with AN (n = 55) which also changed following clinical refeeding (n = 44). The authors also measured specific microbial-derived metabolites and found elevated concentrations of fecal branched-chain fatty acids (BCFAs, products of protein fermentation) in patients with AN which did not return to levels measured in the controls (n = 55) following clinical refeeding [6]. Collectively, these results indicate that the intestinal microbiota of clinically refed patients with AN remains metabolically abnormal.

Mechanisms

Although these studies establish the presence of a dysbiotic intestinal microbiota in patients with AN, the mechanism by which an abnormal enteric microbial community influences either the persistence or the treatment of eating disorders has not yet been fully elucidated. One possible mechanism is via the host immune system within the context of "molecular mimicry," wherein bacteria produce compounds that mimic those native to the host. Auto-antibodies that recognize alpha-melanocyte-stimulating hormone (α-MSH) and contribute to regulation of food intake and behavior have become an intriguing avenue of research into the molecular mechanisms behind disordered eating [83]. Proteomics has revealed that the caseinolytic protease B (ClpB) protein produced by commensal *Escherichia coli* is an antigenic mimic of α-MSH [84]. Mice immunized with bacterial ClpB have lower bodyweights, food consumption, and anxiety than controls, and patients with AN, BN, and BED have elevated levels of plasma ClpB protein [85]. Together, these studies suggest a role for the intestinal microbiota in the initiation or persistence of eating disorders. However, the influence of an eating disorder-associated gut microbiota on its host both prior to and during clinical refeeding is yet to be determined.

Clinical Relevance and Conclusions

Will research on the intestinal microbiota truly yield revolutionary perspectives on illnesses including eating disorders, or will we look back on it as a blind alley in science? Chances are good that the reality will be somewhere in between. Flexible skepticism is a safe stance, but should not impede attempts to detail and clarify the role of the intestinal microbiota in AN and other eating disorders. It is logical to assume that severe alterations in energy consumption and availability (as in AN, BN, and BED) would have effects on the intestinal ecosystem. Living in a competitive environment, intestinal bacteria (and presumably other microorganisms) that are well suited to either a low-energy environment (such as in AN) or a variable-energy environment (such as BN and BED) may be more likely to survive and dominate. Whether dysbioses exist that predispose to extreme appetite imbalance is unknown and is a difficult scientific puzzle whose solution will require prospective studies. More tractable are studies in which we determine whether intestinal dysbioses contribute to persistence, recovery, or relapse from eating disorders. Though it is unlikely that the intestinal microbiota will be the sole therapeutic target in treating AN, it is possible that augmenting treatment with agents that target the intestinal microbiota may facilitate weight gain, decrease GI distress associated with renourishment, and perhaps even reduce anxiety and depression via the brain-gut-microbiota axis. Future work branching beyond AN to the other eating disorders—not only BN and BED, but also perplexing childhood illnesses such as avoidant/restrictive food intake disorder (ARFID) and pica—may expand the clinician's toolbox for treating these debilitating illnesses.

Compliance with Ethical Standards

Conflict of Interest Ian M. Carroll reports a grant from National Institute of Mental Health (R01MH105684). Elaine M. Glenny, Emily C. Bulik-Sullivan, and Quyen Tang report that they are funded from this grant.

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Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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