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Challenges and priorities for researching the gut microbiota in individuals living with anorexia nervosa

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Abstract

Objective: The gut microbiota is implicated in several symptoms and biological pathways relevant to anorexia nervosa (AN). Investigations into the role of the gut microbiota in AN are growing, with a specific interest in the changes that occur in response to treatment. Findings suggest that microbial species may be associated with some of the symptoms common in AN, such as depression and gastrointestinal disturbances (GID). Therefore, researchers believe the gut microbiota may have therapeutic relevance. Whilst research in this field is rapidly expanding, the unique considerations relevant to conducting gut microbiota research in individuals with AN must be addressed.

Method: We provide an overview of the published literature investigating the relationship between the gut microbiota and symptoms and behaviors present in AN, discuss important challenges in gut microbiota research, and offer recommendations for

addressing these. We conclude by summarizing research design priorities for the field to move forward.

Results: Several ways exist to reduce participant burden and accommodate challenges when researching the gut microbiota in individuals with AN.

Discussion: Recommendations from this article are foreseen to encourage scientific rigor and thoughtful protocol planning for microbiota research in AN, including ways to reduce participant burden. Employing such methods will contribute to a better understanding of the role of the gut microbiota in AN pathophysiology and treatment.

Public Significance: The field of gut microbiota research is rapidly expanding, including the role of the gut microbiota in anorexia nervosa. Thoughtful planning of future research will ensure appropriate data collection for meaningful interpretation while providing a positive experience for the participant. We present current challenges, recommendations for research design and priorities to facilitate the advancement of research in this field.

KEYWORDS
anorexia nervosa, eating disorder, gut microbiome, gut microbiota

1 | INTRODUCTION

The underlying mechanisms driving the development and maintenance of anorexia nervosa (AN) are poorly understood (Phillipou et al., 2019), but evidence points to a dynamic interplay of biological, psychological, genetic and environmental factors (Bulik et al., 2022; Kan & Treasure, 2019; Munro et al., 2017; Watson, 2019). Concurrently, treatments that are efficacious in the long term are scant (Murray et al., 2019), and an estimated 30%–50% of individuals diagnosed with AN do not achieve recovery in long-term follow-up (Eddy et al., 2017; Fichter et al., 2017; Keel & Brown, 2010; Smink et al., 2013). The development of effective treatments is urgently needed to improve treatment outcomes and recovery rates.

Exploring novel therapies for eating disorders (ED), including AN, is gaining research attention. Therapies that target the gut microbiota are one such line of inquiry that holds promise due to the hypothesized role of the gut microbiota in the development and maintenance of AN, and co-occurring symptoms (Landini et al., 2022; Wei et al., 2022). The gut microbiota composition is understood to influence various host systems and functions, including energy extraction and appetite, biosynthesis of vitamins, hormones and neurotransmitters, metabolism, and immunity (Lynch & Pedersen, 2016), as well as anxiety, stress, depression, body weight, eating behavior, and GI disturbances (GID) (Boscaini et al., 2022; Dinan & Cryan, 2015; Guinane & Cotter, 2013; Lynch & Pedersen, 2016; Mithieux, 2018; Slyepchenko et al., 2017; van de Wouw et al., 2017). This is of specific relevance for AN, as these host functions are found to be disturbed in individuals with AN (Sudo, 2021; Terry et al., 2022).

The physical environment, including factors such as urban or rural early childhood surroundings and pet ownership, alongside behavioral factors such as diet, exercise, stress, and sleep, all influence the composition and function of the gut microbiota (Cryan et al., 2019). Dietary intake is a key modifier of the microbiota as nutrients and substances from food, particularly fiber, act as substrates for colonic microbial metabolism (Singh et al., 2017). Fat and protein (Jardon et al., 2022), ultra-processed foods (Fernandes et al., 2023), and additives, including emulsifiers and artificial sweeteners (Chassaing et al., 2022; Suez et al., 2022), are other dietary components that influence the gut microbiota. In addition to individual nutrients or dietary components, overall dietary patterns also influence the composition of the gut microbiota (De Filippo et al., 2010; G. D. Wu et al., 2011). Dietary modification can change the gut microbiota rapidly, with extreme dietary changes shifting the microbiome in as little as 24 h (David et al., 2014). For example, the consumption of a fast-food diet (i.e., a diet rich in animal fat and lower in fiber) or a Mediterranean-style diet (i.e., a diet rich in minimally processed foods, including whole grains, vegetables, fruits, nuts, fish and olive oil), was shown to alter the composition of the human gut microbiota in 4 days (Zhu et al., 2020). Importantly, changes in composition are often temporary, and it is unclear whether permanent changes from diet alone are possible (Leeming et al., 2019).

In addition to the composition of the diet, disordered eating behaviors, such as laxative use, energy restriction, and purging, may also influence the composition of the gut microbiota in AN. Untangling the relationship between disordered eating behaviors and compositional changes in the gut microbiota may elucidate mechanistic pathways contributing to AN onset and progression. Furthermore, the gut microbiota may be an effective therapeutic target for nutritional rehabilitation strategies (Landini et al., 2022; Wei et al., 2022).

Recently, several articles detailed the mechanistic importance of the gut microbiota in the etiology of AN and called for further research to inform the development of novel therapies (Dhopatkar et al., 2022; Frostad, 2022; Galmiche et al., 2022; Landini et al., 2022; Reed et al., 2021; Ruusunen et al., 2019; Sirufo et al., 2022; Sudo, 2021; Terry et al., 2022; Wei et al., 2022). However, the issues in conducting this research have yet to be addressed. Therefore, the present paper draws from the authors' experiences in this field to highlight challenges and considerations when investigating the gut microbiota in individuals with AN.

2 | WHY TARGET THE GUT MICROBIOTA IN ANOREXIA NERVOSA?

2.1 | Brief overview of the gut microbiota

"*Gut microbiota*" is the collective term for the trillions of microorganisms that reside within the GI tract, whereas "*gut microbiome*" refers to the genetic material of these microorganisms and hence encompasses their functional potential (i.e., what roles they can perform). Through the metabolism of ingested foods, the microbiota produces metabolites, including neurotransmitters, neuropeptides, short-chain fatty acids (SCFAs), and hormones (de Vos et al., 2022). These metabolites facilitate communication between the gut and the brain via the bi-directional gut-brain axis. Communication from the gut to the brain can occur via neural, immune, and endocrine pathways (Cryan et al., 2019). Communication from the brain to the gut can occur via stress-mediated virulence gene expression and the autonomic nervous system that controls gut function and motility (Margolis et al., 2021). The function of the gut microbiota is far-reaching, and core AN symptoms such as depression, anxiety, and disordered eating behavior have been associated with the composition of the gut microbiota (Kleiman et al., 2015; A. M. Monteleone, Troisi, Fasano, et al., 2021; Terry et al., 2022).

2.2 | Composition of the gut microbiota in individuals with anorexia nervosa

The gut microbiota composition in individuals with AN differs from that of healthy controls and remains so even after inpatient treatment (Di Lodovico et al., 2021; Fouladi et al., 2022). The roles of individual bacterial taxa in symptoms of AN have not yet been fully determined, nor has a microbial signature for AN been identified. However, compared to healthy controls, mounting evidence highlights the greater abundance of species associated with increased energy harvest, such as *Methanobrevibacter smithii* (Armougom et al., 2009; Borgo et al., 2017; Mack et al., 2016; Million et al., 2013); low fat mass, such as *Akkermansia muciniphila* (Mack et al., 2016); decreased appetite, including *Enterobacteriaceae* (Hanachi et al., 2019); and lower abundance of SCFA producers, for example, *Roseburia* and *Eubacterium* (Borgo et al., 2017; Fan et al., 2023; Hanachi et al., 2019; Mack et al., 2016; Morita et al., 2015). Overall diversity (alpha-diversity) may also be lower in individuals with AN compared to controls without AN (Kleiman et al., 2015). Recently, research has extended to the viral gut microbiome where alpha-diversity may be greater in those with AN compared to healthy controls (Fan et al., 2023). In addition, dietary intake and ED behaviors may contribute to its disturbances in individuals with AN (Di Lodovico et al., 2021). Hence, whether AN onset influences gut microbiota composition or whether the gut microbiota leads to the development of AN requires further investigation.

Most recent studies exploring gut microbiota pre-and post-inpatient treatment for AN report some changes in the gut microbiota over time (Kleiman et al., 2015; Mack et al., 2016; A. M. Monteleone, Troisi, Fasano, et al., 2021; P. Monteleone et al., 2019), whilst one study reports no significant differences (Prochazkova et al., 2021). After inpatient treatment, some studies have reported increases in alpha-diversity compared with pretreatment (Kleiman et al., 2015; Mack et al., 2016; A. M. Monteleone, Troisi, Fasano, et al., 2021; Schulz et al., 2021), yet alpha-diversity remains lower than that of healthy controls (Kleiman et al., 2015). Whilst it is unclear whether perturbations in the gut microbiota precede AN diagnosis, data support the noticeable shifts in the gut microbiota after inpatient treatment, suggesting treatment can influence the gut microbiota. Whether these changes may be beneficial for AN treatment outcomes and recovery requires further investigation.

2.3 | Associations between the gut microbiome and eating disorder-related symptomology

The composition of the gut microbiota may be associated with key features of AN, including disorders of gut-brain interaction (DGBI, disorders classified by GI symptoms related to motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota, and altered central nervous system processing) (Drossman, 2016; Dhopatkar et al., 2022), psychological symptoms, and ED psychopathology (Ruusunen et al., 2019). Therefore, altering the composition of the gut microbiota may possibly improve symptoms and treatment outcomes. However, whilst the overall composition of the gut microbiota has been associated with these features, the role of specific taxa within these relationships is unclear. Identifying microbial organisms with mechanistic relevance is of interest to inform the development of targeted therapies such as probiotics (See Garcia et al., 2023 for a recent systematic review of the gut microbiota in AN).

Whilst some associations between the gut microbiota and ED symptomology have been identified, the therapeutic potential of the gut microbiota in AN still needs to be determined. Prebiotics, probiotics, and fecal microbiota transplant (FMT) all have the potential to modify the gut microbiota and have been suggested as possible therapies for AN (Landini et al., 2022; Wei et al., 2022). Based on findings from studies of other psychopathologies (Essali & Miller, 2020; Musazadeh et al., 2022), targeting the gut microbiome may also expedite improvements in psychological symptoms, ED psychopathology, and gut physiology, potentially leading to more rapid and improved

treatment outcomes. Future research will require multi-disciplinary and collaborative large-scale research programs incorporating clinical trials informed by patient priorities. The specific considerations of microbiota research in AN have not been addressed to date.

3 | CHALLENGES IN CONDUCTING MICROBIOTA RESEARCH IN INDIVIDUALS WITH ANOREXIA NERVOSA

Studying the gut microbiota involves several sequential steps, including metadata collection (lifestyle, medications, and other factors known to influence gut microbiota composition), sample collection, stabilization, laboratory handling, sequencing, and bioinformatics. Although recommendations for gut microbiome research exist for the general population (see (Bharti & Grimm, 2021) and (Kim et al., 2017) for detailed summaries), there is a lack of guidance and expert advice when it comes to collecting gut microbiome-related data from individuals with AN. This paper aims to summarize the nuances of gut microbiota research of AN.

Conducting microbiota research in any population with chronic disease is accompanied by logistical and ethical challenges (Ma et al., 2018; Szostak et al., 2022). Research with individuals with AN adds additional complexity since the psychological distress, physical symptoms, and medical complications associated with the disorder may interfere with an individual's willingness and ability to participate (Ortiz et al., 2020). In this section, we discuss challenges in the field, highlighting that, to date, the feasibility and acceptability of microbiota research in this group of individuals are yet to be investigated.

3.1 | Timely sample collection

Collecting a timely stool sample is integral to the precision of gut microbiota data. Therefore, it is critical to adapt sample collection methods to make them feasible for the target participant. Most individuals with AN experience gastrointestinal disturbances (GID) (Riedlinger et al., 2020), including constipation (Sileri et al., 2014). Bowel movements can be infrequent across the spectrum of disorder severity, and constipation will reduce the opportunities to collect a stool sample. The increased attention to bowel habits may induce stress as individuals with AN experience heightened GI-specific anxiety (Brown et al., 2021). We suggest choosing a stool collection method that requires a small amount of stool. For example, a swab and stabilizing preservation agent within a specimen container that allows stabilization of the sample at room temperature. This addresses the logistical barriers of returning a fresh sample immediately to the research team. Furthermore, samples collected with a stabilizing solution have been shown to maintain a stable microbiota, similar to those immediately frozen in −80 degrees (Li et al., 2023). We also suggest protocols include a flexible window for sample collection. Allowing 3 days for sample collection has been used effectively in oncology patients with constipation (Hogue et al., 2019). The unpredictability of bowel motions in individuals experiencing constipation also has consequences for the timing of metadata collection, which should be collected as close to the time of stool collection as possible. To address this, flexible methods, such as online surveys, can be used with participants asked to complete surveys on the same day as the stool sample collection. Alternatively, if other measures require a face-to-face visit with the study team, participants could be encouraged to consume foods or fluids that the participant accepts and will induce a bowel movement for that individual, for example, dried fruit or coffee. Considerations for metadata collection are further discussed in Section 3.2.

3.2 | Collecting metadata

Capturing appropriate metadata is vital for the meaningful interpretation of gut microbiota data (Cernava et al., 2022). Essential metadata should include dietary intake, GI symptoms and stool consistency, psychological symptoms, ED symptoms, and physical activity, as these variables influence the gut microbiota and AN.

3.2.1 | Dietary intake data

Collecting dietary intake information from individuals with AN is a compromise between capturing accurate data and prioritizing the participants' safety. Some existing dietary assessments, such as food records, are time-consuming and burdensome (Shanahan et al., 2021). Individuals with AN often over-report energy intake when using food records (Forbush & Hunt, 2014; Schebendach et al., 2012), and it is unclear whether recording food intake exacerbates ED symptoms. These reasons may explain why only a few studies have characterized the intake of individuals with AN. However, dietary intake is one of the most significant contributors to the composition of the gut microbiota (Singh et al., 2017), and its collection is essential to interpreting findings. Best practice suggests the collection of 3-days of dietary data to capture the natural variation of the habitual diet (Johnson et al., 2020). However, a carefully timed diet recall that captures the dietary intake 24 h before stool sample collection can be sufficient (Shanahan et al., 2021). In this instance, an online 24-h recall platform, such as the Automated Self-Administered 24-h Dietary Assessment Tool (ASA-24), may be useful to allow the participant to complete the recall at home as soon as the sample is collected. Collaborating with nursing and food service staff in the clinical setting may be a useful strategy to facilitate accurate dietary assessment. Whatever the setting, the validity of dietary data are contingent on its collection, coding and analysis by a nutrition professional and dietary data should be verified for accuracy upon collection by the research team (Shanahan et al., 2021).

3.2.2 | Gastrointestinal disturbances and stool consistency

Gastrointestinal disturbances (GID), including the complex symptoms characteristic of DGBI such as irritable bowel syndrome (IBS), are common among individuals with AN (Wiklund et al., 2021) and persist after treatment (West et al., 2021). The severity of GID has been associated with gut microbiome composition in individuals with IBS,

including microbial richness, presence of methanogens and enrichment of Clostridiales or *Prevotella* (Tap et al., 2017). In individuals with AN, the severity of GID has been correlated with an increased abundance of *Dialister*, *Robinsonielle*, and *Enterococcus* (Hanachi et al., 2019). Importantly, stool consistency has been identified as one of the most significant potential confounders affecting markers of microbiome composition (Gacesa et al., 2022; Vandeputte et al., 2016). Given the common occurrence of constipation in individuals with AN, stool consistency may have implications for diversity measures (Vandeputte et al., 2016). Microbial composition is unevenly distributed throughout a sample, especially if it is hard or lumpy. Therefore, if feasible, we suggest stool collection protocols include instructions for participants to mix the sample before collecting it in the tube (W. K. Wu et al., 2019). In addition, measuring GID, particularly stool consistency, is important for interpreting microbiota data in AN. Stool consistency should be measured using the validated Bristol Stool Form Scale (BSFS), a proxy for transit time (Lewis & Heaton, 1997). GID assessment is more challenging as no GID measurement tools have been validated in individuals with AN or any other ED. We recommend that current tools to measure GID be validated for AN, including Visual Analogue Scale-IBS, VAS-IBS (Bengtsson et al., 2007); Gastrointestinal Symptom Score, GISS (Adam et al., 2005), or that novel GID assessment tools be created bespoke for the AN population.

3.2.3 | Psychological symptoms

Comorbid mental health conditions are experienced by the majority of individuals with AN (Blinder et al., 2006) and are associated with an altered gut microbiome (Margolis et al., 2021; McGuinness et al., 2022). Modulating the gut microbiome via probiotic supplementation can induce positive changes in depressive symptoms (Yang et al., 2020). Furthermore, evidence from rodent studies shows that FMT, transferring the fecal matter of a human with depression to a germ-free rodent, can induce symptoms of depression (Kelly et al., 2016), highlighting a mechanistic role of the gut microbiota in depressive symptoms. Therefore, psychological symptoms are an important component of the psychopathology of ED and should be collected in microbiota studies to understand the microbial relevance of the disorder better. Psychological measures, including evaluation of depressive and anxiety symptoms, can be collected using surveys, including online surveys, which help improve accessibility (Ortiz et al., 2020). Online surveys also allow for accommodations to be built into the design; this can include links to resources for further support. Such accommodations have been identified as important components of the research process for individuals with an ED (Ortiz et al., 2020). Commonly used and validated self-report psychological measurement tools include the Depression, Anxiety, Stress Scale, DASS-21, (Henry & Crawford, 2005) and Beck Depression Inventory, BDI, (Wang & Gorenstein, 2013). Both can be administered via online or digital survey platforms, encouraging participants to take short breaks throughout the surveys and providing links to support services. This may help reduce the burden of reporting psychological symptoms.

3.2.4 | Eating disorder behaviors

Eating disorder (ED) behaviors such as restriction, bingeing, and purging may impact the gut microbiota differently. For example, one study of individuals with AN-restrictive type (AN-R) and AN-binge-purging type (AN-BP) reported that the sub-type of AN explained differences in the microbiome composition (Mack et al., 2016). Additionally, another study identified 12 fecal metabolites that differentiated between AN-R, AN-BP, and healthy controls (A. M. Monteleone, Troisi, Serena, et al., 2021). In contrast, a separate study reported no significant differences between AN-R and AN-BP (Morita et al., 2015). Individual ED behaviors (e.g., restriction or laxative use) have also been associated with the type and severity of GID (Wiklund et al., 2021); however, a greater investigation is needed to understand the relationships between ED behaviors and alterations in the gut microbiome. The gold standard for measuring ED symptoms is the Eating Disorder Examination (EDE) semi-structured interview (Fairburn & Beglin, 1994). The EDE requires a trained interviewer and can take between 30 and 60 min to administer. However, disclosing ED behaviors to an interviewer may be distressing (Aardoom et al., 2012), therefore we recommend the EDE-Q, a 28-item self-report version of the EDE as a more feasible alternative. (Aardoom et al., 2012). ED behaviors may have rapid effects on the composition of the gut microbiome. Therefore, capturing behaviors engaged within the 24 h before sample collection is vital, and we recommend simultaneous collection of eating behavior and dietary data.

3.2.5 | Physical activity

Many individuals with AN engage in compulsive exercise to control energy expenditure and maintain low body weight (Rizk et al., 2020). Concurrently, exercise can influence the composition of the gut microbiota (Boytar et al., 2023).

Measurement of physical activity should be incorporated into microbiome research in AN. However, this must be done sensitively to avoid increasing the risk of harmful monitoring of behaviors (Simpson & Mazzeo, 2017; Wons et al., 2022). The mode of measuring exercise must also be carefully considered given individuals with AN might underreport light physical activity (Bezzina et al., 2019). Wearable devices such as accelerometers, that do not provide participants with feedback (e.g., step count or calorie output), are recommended for measurement of duration and intensity of habitual exercise.

Given the number of characteristic features that are core to AN, that also may affect the gut microbiota composition, selecting an appropriate control group is challenging as it is unclear whether dietary, behavioral or psychological aspects may be driving identified differences. Previous observational studies have used healthy controls,

constitutionally thin controls, and athletes as comparator groups. We suggest working with a statistician to consider matching cases and controls or adjusting data for variables known to contribute to AN and gut microbiota composition. Variables to consider include age, sex, BMI, and diet quality.

In summary, several measures are crucial for interpreting microbiome data from individuals with AN. However, this can lead to a high burden for participants due to the need for completing extensive numbers of patient-reported outcome tools. To better understand the role of the gut microbiota in AN, longitudinal studies are needed. However, providing data over multiple time points further increases participant burden. To address this, we recommend collaborating with clinical teams and integrating data collection with routine clinical data.

3.3 | Collaborating with clinical teams

As described above, extensive data collection is important given the intricate relationship between the gut microbiota and physical and psychological health. This requires long questionnaires, which increases the participant burden. Individuals with EDs identify low participant burden as a priority for participating in ED research (Ortiz et al., 2020). We suggest integrating gut microbiota research into clinical settings. Partnering with ED units where routine clinical data are collected to monitor patient progress (e.g., ED symptoms, psychiatric symptoms, medications), and adding measures that are either not routinely collected or not comprehensively measured, but could be mutually beneficial (e.g., stool sample for microbiota analysis, GI symptoms, dietary intake), is a prime opportunity to progress the field. Such a strategy will improve the efficiency of data collection, reduce participant burden, and encourage the development of collaborations and sustainable infrastructure that could be harnessed for use in future research (Garland & Brookman-Frazee, 2015).

3.4 | Ethical considerations

The explosion of scientific research on the gut microbiome has been accompanied by keen interest from the public. Microbiome analysis is readily accessible to those with financial means, and the food industry has capitalized on our collective interest in "gut health" (Ma et al., 2018). Providing individuals with microbiome analysis reports

TABLE 1 Challenges and opportunities in microbiota research in individuals with anorexia nervosa.

Challenges	Research priorities
Stool sample collection	
• Feasibility and acceptability of individuals with AN providing stool sample is unknown. • Constipation leads to difficulty in predictable sample collection and increases logistical challenges (Hogue et al., 2019).	• Establish the feasibility and acceptability of providing stool samples. • Prioritize the use of collection and analysis methods that accommodate small amounts of stool (i.e., a Swab method). • Flexibility in trial timeline to allow for extending sampling periods. • Employ collection kits that contain stabilizer so that stool samples can be kept safely at room temperature until returned to research team.
Dietary intake	
• Approaches to collecting dietary intake data are time-consuming (Shanahan et al., 2021). • In AN, over-reporting may lead to reporting errors (Forbush & Hunt, 2014).	• Development of comprehensive objective measures of dietary intake (e.g., metabolomics). • Characterize nutritional rehabilitation protocols currently used in inpatient clinics, including measurement of macronutrient distribution and micronutrient content.
Measurement of gastrointestinal symptoms	
• Available measurement tools have not been validated for use in individuals with AN.	• Validation of commonly used tools to determine validity for use in AN. • Development of new measurement tools that consider the complexity of GID for individuals with AN (e.g., visceral hypersensitivity, ED behaviors, psychological distress, altered interoception).
Measurement of psychological and eating disorder symptoms	
• Burdensome and repetitive collection of clinical assessments.	• Collaborate with clinical teams to facilitate linking of gut microbiome to routinely collected clinical outcomes. • Use digital or online data collection platforms to improve feasibility.
Participant feedback	
• Some studies provide participants with their microbiome analysis report which is a potential safety concern in AN (Loughman & Staudacher, 2020; Ma et al., 2018)	• Consider provision of feedback of microbiome findings in context of entire sample, rather than individualized feedback. • If participants request access to their individual data, the study team must provide guided interpretation and appropriate support from a dietitian. • Consultation with key stakeholders (experts by experience, dietitians, psychiatrists, psychologists, nurses) to formulate clear and positive messages.

has been debated (Loughman & Staudacher, 2020; Ma et al., 2018). Commercial companies often provide dietary recommendations based on minimal evidence alongside microbiome reports. As a field, we are far from defining a "normal" or "healthy" microbiome, and there is insufficient evidence for specific dietary recommendations based on an individual's microbiome report. Furthermore, if incorrectly interpreted, there is a risk of harm, particularly for individuals with AN who experience increased vulnerability to rigid food rules, fear and anxiety about food, and increased risk of intentional weight loss (Levinson & Williams, 2020). For these reasons, we suggest that individual microbiota profile results from microbiota research are not shared with participants. We also acknowledge that if microbiota results are requested, the research team must provide guided interpretation and appropriate support from a dietitian. Lastly, the lived experience perspective has so far been overlooked. Individuals with AN have not yet been asked about the acceptability of gut microbiome research or interest in this field as a treatment option. We strongly suggest collaboration and qualitative research in this area to inform future practice and guide research questions.

4 | PRIORITIES FOR THE FIELD

In light of the abovementioned considerations relating to sample collection, metadata, clinical collaborations, and ethics, we present research priorities to ensure the field is well-placed to continue producing meaningful gut microbiota data as we work toward therapeutic breakthroughs. Table 1 summarizes the challenges derived from the current literature and research priorities to move the field forward.

5 | CONCLUSIONS

To date, a lack of mechanistic understanding of the development and perpetuation of AN has contributed to the limited efficacy of available treatments. The gut microbiota offers a promising therapeutic target, and research is underway to identify mechanisms to inform new therapies. The field must now move beyond characterizing the microbiota and finding associations, to investigating the underlying mechanisms linking the gut microbiota to AN. We urge researchers investigating the gut microbiota to consider the unique challenges and opportunities when planning and conducting research in individuals with AN. This will ensure safe and ethical data collection, leading to meaningful results that can improve outcomes for people with AN.

AUTHOR CONTRIBUTIONS

Madeline West: Conceptualization; writing – original draft; writing – review and editing. **Susan Hart:** Writing – review and editing. **Amy Loughman:** Writing – review and editing. **Felice Jacka:** Writing – review and editing. **Heidi Staudacher:** Writing – review and editing. **Afrouz Abbaspour:** Writing – review and editing. **Andrea Phillipou:** Writing – review and editing. **Anu Ruusunen:** Writing – review and editing. **Tetyana Rocks:** Writing – review and editing.

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