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Gastrointestinal symptoms, gut microbiome, probiotics and prebiotics in anorexia nervosa: A review of mechanistic rationale and clinical evidence

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ABSTRACT

Recent research has revealed the pivotal role that the gut microbiota might play in psychiatric disorders. In anorexia nervosa (AN), the gut microbiota may be involved in pathophysiology as well as in the gastrointestinal (GI) symptoms commonly experienced. This review collates evidence for the potential role of gut microbiota in AN, including modulation of the immune system, the gut-brain axis and GI function. We examined studies comparing gut microbiota in AN with healthy controls as well as those looking at modifications in gut microbiota with nutritional treatment. Changes in energy intake and nutritional composition influence gut microbiota and may play a role in the evolution of the gut microbial picture in AN. Additionally, some evidence indicates that pre-morbid gut microbiota may influence risk of developing AN. There appear to be similarities in gut microbial composition, mechanisms of interaction and GI symptoms experienced in AN and other GI disorders such as inflammatory bowel disease and functional GI disorders. Probiotics and prebiotics have been studied in these disorders showing therapeutic effects of probiotics in some cases. Additionally, some evidence exists for the therapeutic benefits of probiotics in depression and anxiety, commonly seen as co-morbidities in AN. Moreover, preliminary evidence for the use of probiotics in AN has shown positive effects on immune modulation. Based on these findings, we discuss the potential therapeutic role for probiotics in ameliorating symptoms in AN.

1. Introduction

Eating disorders (EDs), their aetiology and maintenance, including bio-immuno-metabolic causes have come to the forefront of research attention in psychiatry. Factors within the physical and social environment, as well as biological factors such as those within the brain, endocrine, immune and gastrointestinal systems have all been found to contribute to the pathophysiology of EDs (Himmerich et al., 2019). Anorexia nervosa (AN), an eating disorder, is characterised by significantly low body weight, an intense fear of weight gain and body image disturbance. Sufferers exhibit an extreme fear of fatness and demonstrate behaviours to achieve weight loss or maintain a low weight. Their valuation of self is unduly dependent on their body weight and shape. Two subtypes of AN can be distinguished: the restrictive subtype (AN-R), in which severe food restriction is the primary means of losing weight; and the binge-eating/purging subtype (AN-BP), in which restriction is combined with episodes of consuming unusually large amounts of food followed by compensatory behaviour including self-induced vomiting, laxative or diuretic abuse, and/or excessive exercise (American Psychiatric Association, 2013). AN has one of the highest mortality rates among mental health disorders and is associated with morbidity for sufferers (van Hoeken and Hoek, 2020) and their carers (Kyriacou et al., 2008). Prevalence rates range from 0.3 % to 4 % in females and ~ 0.3 % in males (van Eeden et al., 2021).

In AN, self-starvation has physical and mental health consequences, e.g., changes in various organ systems such as the brain and the gut, and changes on cellular and molecular level, such as epigenetic changes. These consequences of self-starvation are important maintaining factors in AN (Himmerich et al., 2019). Therefore, research to identify ways to elucidate and interrupt this cycle that maintains the disorder are...
clinically warranted. The gut microbiome and its role in the gut-brain axis are being studied in relation to mental health disorders by many researchers around the world (Shoubridge et al., 2022). Thus, hypotheses and evidence have been presented for the role of the microbiome in the development and maintenance of AN (Sudo, 2021).

Gastrointestinal (GI) dysfunction in AN is at the heart of not only symptoms suffered but also in the acceptance of nutritional treatment. In this article we consider the interaction between the gut microbiome and AN especially in relation to GI symptomatology, examine available evidence for the role of the microbiome in known GI pathology and the use of modulators such as probiotics and prebiotics as a possible adjunct to current treatment modules.

2. Recent biological findings in AN

Genetic studies over the past 20–30 years have increased our understanding of the biological basis of AN. Heritability has been suggested to account for over 50 % of causality (Bulik et al., 2006). A large multi-centre GenomeWide Association Study has shown significant associations of eight loci with AN (Watson et al., 2019). It is possible that these might shape personality traits, psychological vulnerabilities and possibly interact with familial and societal factors (Himmerich et al., 2019). Recent studies have found genetic links between AN and metabolic disorders (Watson et al., 2019). Additionally, bi-directional associations between AN and auto-immune diseases have been shown (Hedman et al., 2019). Moreover, differences seen in the gut microbiome in AN compared with healthy controls (HCs) has brought the possible role of the gut-brain axis in the causation and maintenance of AN in the spotlight (Seitz et al., 2019), further raising interest in the bio-immuno-metabolic aspect of the pathogenesis model in AN.

2.1. GI symptoms and AN

Restriction of food intake and attempts to compensate for intake can result in profound effects on all the systems of the body including the GI tract. In order to continue functioning with a reduced food intake, the body metabolises energy stores including liver glycogen and visceral fat, via glycoegenolysis and lipolysis (Soeters et al., 2012). A state of starvation also induces gluconeogenesis through breakdown of tissues including muscle and epithelium as a way of supplying energy (Soeters et al., 2012).

Stavation and compensatory behaviours affect the GI system in different ways. Effects may include weakened and/or dysfunctional musculature, reduced or dysfunctional absorptive surfaces, changes in secretion of digestive juices, dilatation of the stomach wall (in the AN-BP subtype) and differences in sphincter function in various parts of the GI tract (Santonicola et al., 2019). A majority of sufferers experience GI symptoms, which may be organic or functional (Salvioli et al., 2013). AN patients may complain of upper GI problems such as dysphagia, heartburn, nausea, vomiting and early satiety. Objective measures of delayed gastric emptying to both solids and liquids have been shown in AN compared with HCs (Riedlinger et al., 2020; Santonicola et al., 2019). Comparing results between AN subtypes (AN-R versus AN-BP), gastric emptying has been shown to be similarly delayed in both (Norris et al., 2016). Multiple case studies of gastric dilatation and subsequent effects such as gastric perforation have been reported in AN-BP (Norris et al., 2016; Riedlinger et al., 2020; Gibson et al., 2021). Symptoms related to functional GI disorders (FGID) are also frequently reported in AN. Functional Dyspepsia was found to be significantly higher in AN compared with HCs including postprandial distress (PDS), fullness and increased intensity and frequency of early satiety (Santonicola et al., 2012). An observational study described 83 % of patients with EDs as having at least one functional GI disorder, with postprandial distress most associated with starvation (Wang et al., 2014).

Constipation is a common lower GI complaint in AN and may be related to poor intake, weakened intestinal musculature, dysfunctional peristalsis due to electrolyte alterations and pelvic floor dysfunction (Santonicola et al., 2019). Laxative use in EDs has also been associated with pelvic floor dysfunction (Abraham and Kellow, 2013; Santonicola et al., 2019). Irritable Bowel syndrome (IBS), consisting of altered bowel habit (diarrhoea and/or constipation) in the presence of abdominal pain has been found in 32–64 % of people with EDs including AN, with a significant association related to a lower body mass index (BMI) (Kress et al., 2018).

The AN sufferer’s initial presentation may be with GI symptoms. In one study, AN patients presenting at a gastroenterology service were older with a longer history of multiple GI symptoms, a substantial delay in being diagnosed with an ED, multiple investigations and admissions compared with those presenting to an ED clinic (Emmanuel et al., 2004). Data from General Practice and hospital databases have shown that in 2 years prior to their diagnosis, patients with EDs were prescribed GI related drugs ~ 2.5 times more often compared with those who were not diagnosed with an ED (Demmler et al., 2020). Patients presenting with a Functional GI disorder and a history of disordered eating were younger, more psychologically distressed, more likely to be female, and more educated than those without a history of disordered eating (Porcelli et al., 1998).

Sufferers may report an ‘intolerance’ to certain foods, for example gluten, resulting in avoidance of food groups that they associate with abdominal pain or discomfort, for example starchy food. Indeed, those diagnosed with coeliac disease are at a higher risk of developing an eating disorder (Mård et al., 2017). Reasons purported are the need to be vigilant with their food, GI symptoms causing worries around eating or weight loss from the onset of coeliac disease being a trigger for AN onset. Coeliac disease and AN may have some commonality in pathogenesis (Mostow et al., 2016).

While nutritional rehabilitation, a cornerstone in the AN recovery process, has been shown to improve most of the GI symptoms suffered (West et al., 2021; Riedlinger et al., 2020), the process of nutritional treatment may worsen symptoms for patients. Dysfunctions of digestive processes such as secretion of enzymes & absorption (Takimoto et al., 2014; Winter et al., 2001) may worsen gut symptoms during refeeding. Previous infrequent consumption of dairy foods can result in secondary lactose intolerance (Szilagyi and Ishayek, 2018). Moreover, the anxiety of consuming feared foods may precipitate GI symptoms (Balmus et al., 2019). While recognising and acknowledging the necessity of hunger and satiety signals is an essential part of recovery, hyper-awareness of ‘fullness’ and discomfort may result in difficulty accepting treatment or an increase in compensatory behaviours. Additionally, some symptoms may continue beyond weight restoration. While overall upper and lower GI symptom scores decreased, most individual upper GI symptoms remained significant after nutritional treatment in one study (Mack et al., 2016). In another study, in both AN-R and AN-BP, oesophageal symptoms continued after weight restoration despite oesophageal manometry being normal (Benini et al., 2010). Furthermore, a study showed an improvement in delayed gastric emptying in AN-R with long term weight restoration but not in AN-BP (Benini et al., 2004). Functional GI symptoms persisted in 77 % of patients at a 12-month follow up in one study (Boyd et al., 2010). A study investigating symptoms in individuals who had been admitted with AN in their adolescence ~ 9 years previously, found odds ratios of 3.6 for gastralgia and 5.3 for gastro-oesophageal reflux (Chapelon et al., 2021).

With multiple possible factors influencing the presence of GI symptoms, it is useful to explore whether there is some merit in the supposition that the gut microbiome and changes within it are part of the pathophysiology of AN.

3. The gut microbiome and its variations

The gut microbiota comprises of a vast number of species of bacteria, archaea, fungi, viruses and eukaryotes, estimated to be over 100 trillion microorganisms (Rinninella et al., 2019). Table 1 provides an overview
of variations and factors influencing an individual’s unique gut microbial composition while Table 2 describes the typical composition in a healthy gut, the predominant phyla being Bacteroidetes and Firmicutes representing over 90% of species isolated, with example species that may be seen in the gut.

### 3.1. Functional attributes of the gut microbiome

#### Table 3

| Phylum                | Families               | Genera                      | Species                        |
|-----------------------|------------------------|-----------------------------|--------------------------------|
| Bacteroides           | Bacteroidaceae (65%)   | Bacteroides                 | Bacteroides fragilis           |
| Fimbicutes            | Lachnospiraceae (11%)  | Roseburia                   | Roseburia infantis             |
| Firmicutes            | Ruminococcaceae (8%)   | Ruminococcus                | Ruminococcus bicornulans       |
| Actinobacteria        | Bifidobacteriaceae (1%)| Butyrobacterium             | Butyrobacterium                |
| Proteobacteria        | Enterobacteriaceae     | Escherichia                 | Escherichia coli               |
| Verrucomicrobia       | Akkermansia (1%)       | Shigella                    | Shigella flexneri              |
| Euryarcheota (< 1%)   | Akkermania (1%)        | Akkermania                  | Akkermania marinaea            |
|                       | Ruminococcus bromii    | Methanobrevibacter smithi   |

* Relative abundance based on a healthy urban population cohort (King et al., 2019). Individuals vary in their microbiota content – for example only ~ 40% of this cohort had Methanobrevibacter smithii, while Bacteroides varied from 0.4% to 98% in individual samples.

with AN (Smitka et al., 2021). Some animal studies have shown evidence of the gut microbiome modulating these appetite and satiety cues. For instance, short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate, products of fermentation by the gut microbiota (mostly from dietary fibre fermentation), may modulate anorexigenic PYY and glucagon peptide 1 (GLP1) release peripherally. They may also affect central appetite mechanisms via glutaminergic and gamma aminobutyric (GABA)-ergic pathways (Smitka et al., 2021; Zhang et al., 2021a; Frost et al., 2014). Some evidence also exists on modulation of ghrelin action by the gut microbiota through inducing secretion of ghrelin or changing expression of ghrelin receptors (Schalla and Stengel, 2018). A randomised controlled trial (RCT) in overweight individuals found that ingestion of an inulin-propionate ester with resulting increased propionate production in the colon in the intervention group was associated with raised PYY and GLP1 levels post-prandially as well as significant reduction in weight gain, intra-abdominal lipid accumulation and insulin resistance in the longer term, compared with the placebo group (Chambers et al., 2015). This signifies the immediate effect of the gut microbiome and their production of SCFAs on satiety signalling together with long term effects on tissue deposition in humans. There is evidence that Enterobacteriaceae such as *E. coli* produce caseinolytic protease B (ClpB), a mimetic of anorexigenic alpha melanocyte stimulating hormone (a-MSH). ClpB has been correlated with AN (Breton et al., 2016). Such an interplay of anorexigenic and orexigenic signals may be one of the ways in which the gut microbiome influences pathogenesis or maintenance of AN.

### 3.1. Functional attributes of the gut microbiome

#### Table 3

| Main microbiota phyla in the gut (Rinninella et al., 2019) | Microbiota, their genome and their environment (Qian et al., 2020) | Factors affecting gut microbiota (Dabrowska and Witkiewicz, 2016; Rinninella et al., 2019) | Enterotypes (Costea et al., 2018) |
|-----------------------------------------------------------|------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------|
| Bacteroidetes                                             | Microbiota, their genome and their environment                   | Gestational age, mode of birth                                                      | Stratification of gut microbiota into clusters by predominance of taxa |
| Firmicutes                                                |                                                                  | Age, gender, ethnicity                                                             | Controversial as a reductionist view, but may be helpful in getting an overall sense of the microbiota in communities |
| Actinobacteria                                            |                                                                  | Breastfeeding/complementary feeding                                                | Enterotypes commonly described include: |
| Proteobacteria                                            |                                                                  | Habitual diet and short-term changes in diet                                       | - Bacteroides predominant |
|                                                            |                                                                  | Antibiotics                                                                      | - Prevotella predominant |
|                                                            |                                                                  | Frequency of exercise                                                             | - Firmicutes predominant |
|                                                            |                                                                  | BMI                                                                               | |
|                                                            |                                                                  | Host genetics                                                                     | |
|                                                            |                                                                  | Gut disease                                                                       | |

| Microbiota diversity (Qian et al., 2020) | Microbiota diversity (Qian et al., 2020) | Microbiota diversity (Qian et al., 2020) |
|----------------------------------------|----------------------------------------|----------------------------------------|
| α diversity: diversity of species/strains within a sample (e.g. within an individual) | β diversity: diversity of species/strains between samples (e.g. between different individuals or the same individual over time) | Enterotypes (Costea et al., 2018) |

#### 3.1.1. Energy availability and tissue deposition

An interaction that may have particular significance in AN is the interaction of the gut microbiome and their production of SCFAs on satiety. For instance, short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate, products of fermentation by the gut microbiota (mostly from dietary fibre fermentation), may modulate anorexigenic PYY and glucagon peptide 1 (GLP1) release peripherally. They may also affect central appetite mechanisms via glutaminergic and gamma aminobutyric (GABA)-ergic pathways (Smitka et al., 2021; Zhang et al., 2021a; Frost et al., 2014). Some evidence also exists on modulation of ghrelin action by the gut microbiota through inducing secretion of ghrelin or changing expression of ghrelin receptors (Schalla and Stengel, 2018). A randomised controlled trial (RCT) in overweight individuals found that ingestion of an inulin-propionate ester with resulting increased propionate production in the colon in the intervention group was associated with raised PYY and GLP1 levels post-prandially as well as significant reduction in weight gain, intra-abdominal lipid accumulation and insulin resistance in the longer term, compared with the placebo group (Chambers et al., 2015). This signifies the immediate effect of the gut microbiome and their production of SCFAs on satiety signalling together with long term effects on tissue deposition in humans. There is evidence that Enterobacteriaceae such as *E. coli* produce caseinolytic protease B (ClpB), a mimetic of anorexigenic alpha melanocyte stimulating hormone (a-MSH). ClpB has been correlated with AN (Breton et al., 2016). Such an interplay of anorexigenic and orexigenic signals may be one of the ways in which the gut microbiome influences pathogenesis or maintenance of AN.

### 3.1.2. Appetite and satiety cues

Another interaction of relevance is the gut microbiome’s influence on appetite and satiety cues. AN has been shown to be associated with elevated levels of total and other forms of ghrelin such as the acyl and the des-acyl form (Seidel et al., 2021). Despite the orexigenic nature of ghrelin, AN patients report a reduced appetite. Raised levels of anorexigenic peptide tyrosine tyrosine (PYY) have also been associated

#### Table 2

| Microbiota diversity (Qian et al., 2020) | Microbiota diversity (Qian et al., 2020) | Microbiota diversity (Qian et al., 2020) |
|----------------------------------------|----------------------------------------|----------------------------------------|
| α diversity: diversity of species/strains within a sample (e.g. within an individual) | β diversity: diversity of species/strains between samples (e.g. between different individuals or the same individual over time) | Enterotypes (Costea et al., 2018) |

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Table 3
List of potential gut microbiome-host interactions and their relevance to AN. For further information see text and the cited literature.

| Interaction with the host | Possible effects | Relevance to AN |
|--------------------------|------------------|-----------------|
| **Physical presence of a ‘healthy’ microbiome** | - Pathogens prevented from colonising | - Potential resistance to dysbiosis |
| **Dietary fibre and resistant starch from host used as an energy source by gut microbes, producing short chain fatty acids (SCFAs) – acetate (50-60%), propionate (20-25%), butyrate (15-20%) (Bakker et al., 2015)** | - Energy source for local cells (for example colonocytes) | - Energy extraction from dietary intake of dietary fibre |
| | - Influence on adipose deposition, tissue deposition and weight gain (Delzenne et al., 2011) | - Influencing ability to gain weight during nutritional rehabilitation |
| | - Immune modulation and anti-inflammatory effect by butyrate producers | - Reduction of intestinal permeability by butyrate producing bacteria – an anti-inflammatory effect |
| | - Increased mucus production enhancing barrier function | - Modifying gene regulation of cell lines that are particularly affected in AN (for example the haematopoetic cell lines) |
| | - Inhibition of histone deacetylases, action as ligands to G protein coupled receptors – signalling haematopoetic and non-haematopoetic cell lines, trans- | - Anti-inflammatory effect of SCFAs may counter the pro-inflammatory profile seen in AN |
| | | scriptation factors & modifying gene regulation |
| | - Histone deacetylase inhibition promoting an anti-inflammatory cell phenotype | | |
| | - Epigenetic effect | | |
| | - Mononuclear cells and neutrophils exposed to SCFAs resulting in inactivation of pro-inflammatory NF-kB and down regulation of TNF (Rooks and Garrett, 2016) | | |
| | - Effect on osteoclasts including inhibition of their cell differentiation, thus protective of bone mass (Cu HMS, 2019) | | |
| | - Butyrate can affect energy expenditure and promote thermogenesis in brown and white adipose tissue (Zhang et al., 2021a) | | |
| | - Maturation and differentiation of microglia in the nervous system (Rooks and Garrett, 2016) | | |
| | - Modulation of appetite via central mechanisms including influencing glutaminergic and GABAergic neurons (Smitska et al., 2021) as well as peripheral mechanisms, for example butyrate stimulating the anorexigenic PYY and GLP1 (Zhang et al., 2021a) acting via vagal nerve afferents | | |
| **Dietary tryptophan used by microbes (for eg: Lactobacilli) producing metabolites that are ligands to aryl hydrocarbon receptor (AHR) – transcription factor expressed by immune cells and epithelial cells (Rooks and Garrett, 2016)** | - AHR activation related to normal intestinal epithelial cell barrier function, normalised bacterial load in the lumen | - Anti-inflammatory properties may counter the pro-inflammatory profile seen in AN as well as possible effect on reducing associated GI symptoms |
| | - Resistance to colonisation by pathogens via sequestration of metal ions | - Improved tight junction function may reduce gut permeability and improve gut symptoms |
| | - Reduced mucosal inflammation | | |
| | - Modulation of host cell proliferation including intestinal epithelial cells | | |
| | - Stimulate production of intercellular tight junction proteins (occludin, zonula occludens 1, E-cadherin) | | |
| | - Modulation of immune function including function of macrophages, T cells, production of cytokines, anti-inflammatory effects, production of mucus | | |
| | - Low levels related to neuro-degenerative diseases | | |
| | - Dysregulated metabolism related to carcinogenesis (Rooks and Garrett, 2016) | | |
| | - Methane can slow colonic transit (Lee et al., 2013) | - Improved tight junction function may reduce gut permeability and improve gut symptoms |
| **Gases:** | - H2S acts as source of energy to colonocytes, but in excess can inhibit colonocyte respiration and increase expression of inflammatory genes (Beaumont et al., 2016) | | |
| | - H2 can have an anti-oxidant, anti-inflammatory & anti-apoptotic effect | | |
| | - H2 can cross the blood brain barrier and have a neuroprotective effect (Ontopic, 2018) | | |
| | - Potential modulation of local signalling by neurotransmitters in the intestinal lumen | | |
| | - GABA can inhibit lower oesophageal sphincter relaxation, affect gastric emptying and secretion, intestinal motility and nociception | - Anti-inflammatory properties may counter the pro-inflammatory profile seen in AN as well as possible effect on reducing associated GI symptoms |
| | - GABA may have an anti-inflammatory effect by reducing release of inflammatory cytokines and promoting T-reg cells (Aueri et al., 2015) | | |
| | - Serotonin can affect gut motility including gastric emptying and colonic transit and visceral pain (Vano et al., 2015; Spohn and Mawe, 2017; Strandwitz, 2018) | | |
| | - Serotonin can have a pro- or an anti-inflammatory effect possibly dependent on luminal conditions | | |
| | - Serotonin associated with neurogenesis and the protection of enteric neurons (Spohn and Mawe, 2017) | | |
| | - Microbes can produce or influence the release of neurotransmitters such as GABA and serotonin (Strandwitz, 2018) | - Inflammatory response reduced – likely reducing GI symptoms this can cause |
| | - Serotonin (5 hydroxy tryptamine SHT) production by colonocytes in response to spore producing bacteria | - Slow intestinal transit may result in constipation |
| | - Bacterial enzymes affecting SHT metabolism (Rooks and Garrett, 2016) | - Constipation and colonic gas production may cause feelings of fullness and bloating |
| **Neurotransmitters:** | - Dopamine and norepinephrine can stimulate growth of microbes such as E. coli | - If associated with a high protein diet, and changes in pH, H2S production could have a deleterious effect on the colonocytes and may be associated with inflammatory changes (Blachier et al., 2021; Beaumont et al., 2016) |
| | - Microbes can produce or influence the release of neurotransmitters such as GABA and serotonin (Strandwitz, 2018) | - Effect of stress in changing the gut microbiome in AN |
| | - Serotonin (5 hydroxy tryptamine SHT) production by colonocytes in response to spore producing bacteria | - GABA inhibition of lower oesophageal sphincter relaxation may ameliorate reflux symptoms in AN |
| | - Bacterial enzymes affecting SHT metabolism (Rooks and Garrett, 2016) | - GABA may have an inhibitory effect on visceral hypersensitivity, with potential to ameliorate abdominal pain/hyperawareness experienced in AN |
| | - GABA inhibition of lower oesophageal sphincter relaxation may ameliorate reflux symptoms in AN | - Anti-inflammatory effect of GABA or serotonin may counter the pro-inflammatory profile seen in AN |
| | - Serotonin can improve delayed gastric emptying and delayed colonic transit, but may exacerbate abdominal pain by increasing awareness of pain in AN | - Serotonin could improve delayed gastric emptying and delayed colonic transit, but may exacerbate abdominal pain by increasing awareness of pain in AN |

(continued on next page)
### Table 3 (continued)

| Interaction with the host | Possible effects | Relevance to AN |
|--------------------------|-----------------|-----------------|
| LPS produced by gram negative bacteria | - Activation of Toll like receptors with resulting cytokine cascades (Candelli et al., 2021) | - Inflammatory reaction in the gut epithelium may cause symptoms such as abdominal pain and change in bowel opening. | Possible effect on inflammation and adipose tissue deposition (Delzenne et al., 2011) |
| Appetite and satiety signalling | - SCFAs may stimulate GLP1 and PYY and modulate ghrelin action | - Autoantibodies to various orexigenic and anorexigenic peptides including appetite – this may have a role in pathogenesis or maintenance of AN |

**Note:** NF-kB: nuclear factor kappa B; TNF: Tumour necrosis factor; H₂S: hydrogen sulphide; H₂: hydrogen gas; CH₄: methane; GABA: gamma aminobutyric acid; LPS: lipopolysaccharide; GLP 1: Glucagon-like Peptide 1; PYY: peptide tyrosine tyrosine; αMSH: alpha Melanocyte Stimulating Hormone; T-reg cells: T regulator cells.

### 3.1.3. Gut microbiome in depression and anxiety

The gut-brain axis is said to play an important role in mood and anxiety disorders (Shoubridge et al., 2022) and is being extensively researched. While a comprehensive analysis of this topic is beyond the scope of this review (some reviews on this subject include Cryan et al., 2019, Foster and Mcvey Neufeld, 2013 and Yang et al., 2020), the following sections provide a brief overview of the interaction between the gut microbiome and these conditions given that both mood and anxiety disorders often co-exist with AN.

#### 3.1.3.1. Evidence through animal studies in depression and anxiety

Gut dysbiosis has been associated with depression, stress and anxiety in animal studies. Some changes in microbial abundances include increased Bacteroidetes, reduced Firmicutes in depression and reduced Bacteroides and increased Clostridium species in stress (Lach et al., 2018). Exposure to gut microbiota during a critical interval in early growth can determine whether normal stress responses develop adequately (Foster and Mcvey Neufeld, 2013). FMTs from depressed mice and human participants into GF mice can induce depressive symptoms in recipients implying causality (Zheng et al., 2016; Kelly et al., 2016). Pathogens such as Campylobacter have been correlated to an increased anxiety response in animals while Bifidobacteria and Lactobacillus have been associated with a reduced anxiety response, reversal of depression and resilience to depression-inducing stress (Foster and Mcvey Neufeld, 2013; Cryan et al., 2019; Yang et al., 2020).

#### 3.1.3.2. Evidence through human studies in depression and anxiety

Evidence of gut dysbiosis in human studies includes higher abundance of Enterobacteriaceae, Alstotypes and Bacteroidales and lower numbers of Lachnospiraceae and Faecalibacteria in depression (Yang et al., 2020) and increased Escherichia, Shigella, Fusobacterium and Ruminococcus gnavus abundance in generalised anxiety disorder compared with HCs (Jiang et al., 2018). Healthy women with Prevotella predominant gut microbiome clusters showed significant negative affect and activation of emotion regulating centres in the hippocampus when exposed to negatively valanced images when compared with those with Bacteriodes predominant clusters implying microbiome associated vulnerability to depression (Tillisch et al., 2017). Changes in abundances seen above including increased abundance of Enterobacteriaceae and reduced Lachnospiraceae and Faecalibacteria have also been observed in AN (Section 5) suggesting common gut microbiome related mechanisms. Prevotella predominance in the gut microbiome has been associated with increased weight loss in overweight and in healthy individuals compared with Bacteriodes predominance (Christensen et al., 2019; Hjorth et al., 2019; Zou et al., 2020) suggesting a link between gut microbiome features and vulnerability to AN similar to the link between gut microbiome and vulnerability to depression.

#### 3.1.3.3. Mediators between the gut microbiome and the gut-brain axis in depression and anxiety

Microbial products such as SCFAs may mediate the interaction between the gut microbiota and the gut-brain axis in depression and anxiety. Animal studies have shown anxiolytic effects, mitigation of psychological stress-induced reduction of reward seeking behaviour and restoration of innate anxiety response with SCFA supplementation (van de Wouw et al., 2018; Wu et al., 2022). Higher serum SCFA levels have been associated with attenuated cortisol levels in a human study implicating their effect on the hypothalamic-pituitary-adrenal axis as a mechanism of action (Dalile et al., 2020). Other mechanisms may be modulation of GLP1, PYY or cholecystokinin (CCK) secretion or receptor expression acting peripherally or centrally to influence anxiety-like behaviour (Lach et al., 2018) or via modifying immune responses peripherally or centrally (Fung et al., 2017).

Thus, the gut-brain axis and its interaction with the gut microbiome presents us with multiple sources of evidence, some implying causality...
or vulnerability to a mental health disorder, others suggesting changes as a result of the disorder, implying potential as a maintaining factor. Reversal of or resilience to these disorders through changes in the gut microbiome also present the possibility of microbiome-based treatments as adjuncts to current management.

3.1.4. Gut microbiome and GI symptoms

Looking specifically at GI symptoms, local signalling via neurotransmitters such as serotonin or GABA produced either by microbes or by colonoocytes in response to microbes may influence gut motility as well as sensation of visceral pain (Strandwitz, 2018; Yano et al., 2015). SCFAs or secondary bile acids are likely signalling molecules affecting the release of serotonin from enterochromaffin cells. Serotonin can affect GI secretion and peristalsis (Strandwitz, 2018). A recent study in mice showed that lipo-polysaccharide (LPS), a product of gram-negative bacteria, is associated with reduced serotonin selective reuptake transporters, resulting in raised mucosal serotonin, increased faecal water content and visceromotor responses in the colon (Gao et al., 2022). This indicates one of the ways in which the gut microbiota may influence gut symptoms. GABA has been shown to be secreted by a number of bacteria including some Lactobacilli and Bifidobacteria. Altered GABAergic transmission can affect intestinal motility, gastric emptying, acid secretion and nociception. An engineered strain of Bifidobacterium able to over-express GABA was shown to reduce visceral pain sensitivity in a rat model (Strandwitz, 2018). Thus, GI symptoms in AN may be influenced by the host gut microbial composition through the modulation of local neurotransmitters such as serotonin and GABA.

It has been hypothesised that microbes influence host behaviour including producing symptoms in order to direct the host towards ingesting food facilitating their proliferation and suppressing competitors’ growth (Alcock et al., 2014). GI symptoms can also be a result of microbial metabolism. Methane, a by-product of fermentation, by slowing colonic transit can aid efficient extraction of energy from colonic content. Slow transit may then result in constipation (Lee et al., 2013).

Gut symptoms may also be affected by changes in permeability and inflammatory responses. Gut epithelial integrity and inflammatory response modulation has been associated with various gut microbes. LPS has been shown to increase permeability and the inflammatory response in the gut (Deldenne et al., 2011) as have antibiotics in mice (Feng et al., 2019). Mucin-degrading bacteria (Genus Prevotella) in activity-based rodent models of AN were associated with increased permeability (Achamrah et al., 2019). In contrast, Faecalibacterium prausnitzii and Ruminococaceae have been associated with reduced gut permeability and an immune-protective effect (Morkel et al., 2018) again raising the potential of microbiome-based treatment options in ameliorating GI symptoms in AN.

4. GI disorders and the gut microbiome

As there are commonalities between some GI pathologies and AN (see Table 4), exploring the gut microbiome in these conditions may give an indication of its influence on GI symptoms in AN.

4.1. Inflammatory Bowel Disease (IBD) and the gut microbiome

One hypothesis for the pathogenesis of IBD, a chronic inflammatory condition of the gut with main sub-types Crohn’s disease and Ulcerative colitis, involves an aberrant immune response to an environmental stimulus, such as the gut microbiota, in genetically susceptible individuals (Xavier and Podolsky, 2007). Multiple animal studies have contributed to this hypothesis including an inability for genetically susceptible mice to develop colitis in a germ-free environment, faecal transfer from diseased mice or humans to healthy mice resulting in colitis and the transfer of CD4 lymphocytes from healthy mice to those lacking these lymphocytes transferring ability to induce colitis (Glassner et al., 2020). Studies comparing the gut microbiome in IBD and HCs have found significant differences including reduced diversity, decreased Firmicutes and increased Proteobacteria in IBD (Nishida et al., 2018). Ruminococcus gravis has been positively correlated with Crohn’s Disease and Roseburia species (Family Lachnospiraceae) and Faecalibacterium prausnitzii (Family Ruminococcaceae) have been negatively correlated with IBD presence and severity (Glassner et al., 2020). IBD is also associated with a pro-inflammatory state via cytokines such as interleukin (IL-) 6, 8, 12, 23 and tumour necrosis factor alpha (TNF-α), and a reduced regulatory response via T regulator (T-reg) cells and IL-10 (Yan et al., 2020). Roseburia species have been related to a positive impact on T-reg cells, the secretion of IL-10 and the upregulation of antimicrobial peptides and gut barrier function (Patterson et al., 2017). Therefore, a reduction in Roseburia abundance in the gut microbiome could be related to an abnormal immune response.

4.2. Functional GI disorders (FGIDs) including Functional Dyspepsia (FD) and IBS and the gut microbiome

The possible factors causing and maintaining FD such as sensori-motor abnormalities, altered epithelial barrier function and immune response abnormalities may all be influenced by the gut microbiome. Contributors may be the effects of the microbiome on GLP1, PYY and ghrelin, their ability to impact on neuronal transmission via modulating neurotransmitters such as serotonin or GABA and ability to modulate the inflammatory milieu and epithelial permeability (see Table 3). There is some evidence directly linking FD and dysbiosis such as increased Proteobacteria, reduced Bacteroidetes, Prevotella and Veillonella. Streptococcus abundance has been positively correlated with FD symptoms and Prevotella negatively correlated with symptom severity (Zhou et al., 2022).

Various causative mechanisms are proposed for IBS including abnormal intestinal transit and intraluminal stimuli (including gut microbial products) resulting in mucosal inflammation, changed permeability and increased response to stimuli resulting in visceral pain (Camilleri, 2013) all of which could be impacted on by the gut microbiome. The association of IBS symptoms with stress implies a close brain-gut connection in its pathogenesis (Qin et al., 2014) which can also be modulated by the gut microbiome. Thus, changes in the gut microbiome could affect the presentation of IBS. A review found an overall increase of Enterobacteriaceae and a decrease of genera Faecalibacterium and Bifidobacterium in IBS compared with HCs (Wang et al., 2020).

4.3. Coeliac disease and the gut microbiome

Coeliac disease can present at any point during the lifetime of an individual suggesting that environmental factors such as a change in gut microbiome may be involved in triggering an immune mediated enteropathy to gluten (a protein present in wheat, rye and barley). Gut microbiome changes associated with coeliac disease include increased Proteobacteria, Enterobacteriaceae, Staphylococcus, Bacteroides, Prevotella, and reduced Bifidobacteria and Lactobacilli (Wacklin et al., 2013; Akobeng et al., 2020). These may be a response to the pathogenic process but also may maintain the GI pathology and symptoms through effects on inflammation, visceral sensation and gut transit.

4.4. GI disorders and AN – commonalities

Table 4 compares characteristics of some GI disorders with AN. A Swedish cohort was found to have bi-directional associations between AN and IBD (Hedman et al., 2019), as has a review of case studies, finding the co-existence of AN and Crohn’s disease being the most common (Ilzarbe et al., 2017). In a Danish sample aged 8–32 years, a significant risk of IBD was seen after a diagnosis of AN (Relative Risk (RR) for Crohn’s disease = 1.60; RR for Ulcerative Colitis = 1.66)
Inflammatory Bowel Disease (IBD) (ulcerative colitis (UC) and Crohn’s disease (CD))
- Mechanism of aetiology: Genetic susceptibility + environmental trigger (such as change in the gut microbiome) → aberrant inflammatory response in the gut
- Dysbiosis may maintain inflammation
- Gut microbiota and metabolite findings:
  - ↓ diversity during active disease
  - ↑ Proteobacteria and Enterobacteria
  - ↓ butyrate producing *P. praeurnitzii*, *Roseburia* species
  - Dysregulation of bile acid metabolism, ↑ SCFAs, change in amino acid levels, sphingolipids, polypeptides in faecal samples. (Glossner et al., 2020; Nishida et al., 2018; Khan et al., 2019; Lavelle and Sokol, 2020)

- Evidence for use of probiotics and prebiotics
  - Most effective: 1) Multi-strain probiotics (eg: *Lactobacilli, Bifidobacteria* and *Streptococci*) 2) 12–16 week duration of intervention 3) in UC (Shen et al., 2014; Freidlis et al., 2020; Oka and Sartor, 2020; Zhang et al., 2021b)
  - Prebiotics: No effect on remission of IBD (Wedlake et al., 2014; Benjamin et al., 2011; Zhang et al., 2021b)

- Commonalities and associations with AN
  - Associations between IBD and AN (Hedman et al., 2019; Larsen et al., 2021)
  - Co-existence of AN & Crohn’s disease (Ilizarova et al., 2017)
  - ↑ Pro-inflammatory profile in AN (Dalton et al., 2018)
  - Associations between anti-immune diseases and AN (Watson et al., 2019)
  - Dysbiosis associated with AN may be a possible result of dietary changes, inflammatory changes, or both
  - Common gut microbiome changes:
    - Bacteroidetes: Firmicutes ratio & butyrate producing bacteria such as *Roseburia* spp & Faecalibacterium prunumizii
  - FD symptoms associated with AN (Santonicola et al., 2012)
  - PDS associated most with starvation in EDs (Wang et al., 2014)
  - Upper GI symptoms persisted despite nutritional restoration (Mack et al., 2016); FGID symptoms ± probiotic (mainly with *Lactobacilli* strains) and duration of > 8 weeks → better results (Dale et al., 2019; Sun et al., 2020; Ford et al., 2018; Fatahi et al., 2022)

Functional Dyspepsia (FD): post-prandial distress syndrome (PDS) and epigastric pain syndrome (EPS)
- Aetiology + maintenance mechanisms include: visceral hypersensitivity, gastric sensorimotor abnormalities, immune activation, epithelial barrier permeability alteration, stress, post-infection inflammation, disordered duodeno-gastric feedback, low grade duodenal inflammation, neuronal hyperexcitability with a background of genetic susceptibility (Tziatzios et al., 2020; Zhou et al., 2022; Wauters et al., 2020)
- Gut microbiota and metabolite findings:
  - ↑ Helicobacter pylori
  - SCFAs modulate duodenal bicarbonate secretion
  - E.coli → LPS → delayed gastric emptying → ↑ symptoms (Tziatzios et al., 2020)
  - ↑ Streptococcus and total bacterial load in the duodenal mucosa → ↑ symptoms (Wouters, 2020)
  - ↑ Proteobacteria, ↑ Bacteroidetes, Prevotella, Veillonella (Zhou et al., 2022)

- Probiotics:
  - *Lactobacillus gasseri* → ↓ PDS symptoms (Igarashi et al., 2017)
  - probiotic (Bacillus species) vs. placebo without proton pump inhibitors → ↓ FD symptoms (Wauters et al., 2021)
  - multistrain *Lactobacilli* → ↓ PDS symptoms (Drago et al., 2021)
  - *Lactobacillus rhamnosus* + hydrolysed formula → ↓ risk of developing FD symptoms compared with hydrolysed formula on its own in children with cow’s milk allergy (Nicerino et al., 2019)

- Dysregulation of bile acid metabolism, ↓ Firmicutes, change in amino acid levels, ↑ SCFAs modulate duodenal bicarbonate secretion *E.coli* → ↓ delayed gastric emptying → ↑ symptoms of FD (Wauters et al., 2020)

Irritable Bowel Syndrome
- Aetiology + maintenance mechanisms include: Abnormal gut transit, visceral hypersensitivity to stimuli resulting in hypervigilance of gut function, response to stress, abnormal immune response to dysbiosis (Camilleri, 2013)
- Gut microbiota and metabolite findings:
  - ↔ or ↓ Diversity compared with controls
  - ↑ Bacteroidetes: Firmicutes ratio, ↑ Bacteroides
  - ↑ Enterobacteriaceae (phylum Proteobacteria) & ↑ or ↓ Bifidobacteria
  - ↓ Faecalibacterium (Wang et al., 2020; Pittayanon et al., 2019)

- Probiotics:
  - Trend towards ↓ symptoms
  - Multi-strain probiotic (mainly with *Lactobacilli* and *Bifidobacteria* strains) & *Streptococcus, Bacillus, Enterococcus* strains) and duration of > 8 weeks → better results (Dale et al., 2019; Sun et al., 2020; Ford et al., 2018; Fatahi et al., 2022)
  - low FODMAP diet (with low levels of prebiotics) → ↓ IBS symptoms (Whelan and Staudacher, 2022)

- Coeliac disease
- Aetiology + maintenance mechanisms: genetic susceptibility + trigger (eg gut microbiome change) → immune mediated enteropathy triggered by gluten (a protein found in wheat, barley & rye).
- Gut microbiome and metabolite findings:
  - ↑ Proteobacteria, Enterobacteriaceae, *Klebsiella*, *Staphylococcus*
  - ↓ Firmicutes, *Streptococcus* (Wacklin et al., 2011)
  - No significant difference in asymptomatic coeliac disease compared to controls (Wacklin et al., 2013)
  - ↑ Bacteroides, Prevotella and ↓ *Bifidobacteria, Lactobacilli* (Akobeng et al., 2020)
  - Significantly different gut microbiota in genetically susceptible children compared with controls (Akobeng et al., 2020)

- Evidence for use of probiotics and prebiotics
  - IBS symptoms have been associated with AN (Kress et al., 2018; Kessler et al., 2020)
  - Both IBS and AN have been associated with anxiety and stress (Bin et al., 2014; Zamani et al., 2019; Guarda et al., 2015)
  - Common gut microbiota features include: ↑ Proteobacteria, Enterobacteriaceae & ↓ Faecalibacterium

- Commonalities and associations with AN
  - Significant bi-directional associations between coeliac disease and AN
  - AN after diagnosis of coeliac disease – hazard ratio: 1.46
  - Coeliac disease after diagnosis of AN – odds ratio: 2.18 (Mavrid et al., 2017)
  - Common gut microbiota changes: ↑ Proteobacteria & Enterobacteriaceae
  - To note, most microbiota evidence in coeliac disease is from mucosal sampling compared with faecal sampling in AN

IBD: inflammatory bowel disease; FGID: functional gastrointestinal disorders; FD: functional dyspepsia; PDS: post-prandial distress syndrome; EPS: epigastric pain syndrome; IBS: irritable bowel syndrome; SCFAs: short chain fatty acids; LPS: lipopolysaccharide; FODMAP: fermentable oligosaccharides disaccharides monosaccharides and polyols.

Table 4
Summary of characteristics, gut microbiome findings, use of probiotics and prebiotics in some GI disorders and similarities with AN.
(Larsen et al., 2021). As discussed previously, FD and IBS symptoms have been correlated with AN (Santonicola et al., 2012; Wang et al., 2014; Kress et al., 2018). A bi-directional association has been found between coeliac disease and AN (Märdh et al., 2017).

A meta-analysis of cytokine levels has found increased pro-inflammatory markers associated with AN including IL-6 and TNF α (Dalton et al., 2018). The immune profile seems to be different in AN as compared with primary undernutrition (Gibson and Mehler, 2019) indicating specific mechanisms in play in AN not merely related to starvation. AN has also been associated with other auto-immune diseases (Watson et al., 2019), so it is feasible that an immune dysfunction may be related to dysbiosis and GI symptoms in AN.

As discussed previously, AN patients often present with symptoms related to FGIDs involving FD and IBS. AN is correlated to anxiety and mood disorders (Guarino et al., 2015) as is IBS to stress and anxiety and affective disorders (Qin et al., 2014; Zamani et al., 2019). FGID and AN have been related to immune function abnormalities (Camilleri, 2013; Tzitizios et al., 2020; Dalton et al., 2018). Therefore, there may be similar underlying mechanisms of GI symptoms for both AN and FGIDs.

5. AN and the gut microbiome

Studies investigating the gut microbiome in AN are described in Table 5. They indicate significant dysbiosis in AN compared with HCs. Alpha diversity in AN showed varied results. Some individual studies showed lower diversity (Kleiman et al., 2015; Monteleone et al., 2021a) or similar diversity in AN compared with HCs (Borgo et al., 2017; Mack et al.; 2021b) whereas others found higher alpha diversity including in an individual study (Prochazkova et al., 2021) and a pooled analysis of 4 studies (Di Lodovico et al., 2021).

Regarding abundances of individual taxa, higher abundances of M. smithii (Armougom et al., 2009; Borgo et al., 2017; Mack et al., 2016; Million et al., 2013), mucin-degrading bacteria (Hanachi et al., 2019; Mack et al., 2016; Monteleone et al., 2021a) and lower abundances of anaerobes including butyrate-producing Roseburia, Eubacterium, Anaerostipes and Faecalibacterium (Borgo et al., 2017; Hanachi et al., 2019; Kleiman et al., 2015; Mack et al., 2016; Prochazkova et al., 2021) were found in AN compared with HCs. Additionally, increased abundances of potential pathogens including E. coli (Million et al., 2013), Salmonella & Klebsiella (Hanachi et al., 2019) were also seen. In the pooled analysis, a large effect size for increased abundance of Alistipes & Parabacteroides, and decreased abundance of Roseburia was seen in AN compared with HCs. Furthermore, a medium size effect was also found with increased abundance of Clostridium xidvii, Akkermansia & Eisenbergiella and reduced abundance of Ruminococcus in AN compared with HCs. Roseburia & Anaerostipes were significantly correlated to BMI (Di Lodovico et al., 2021).

Studies comparing AN-R and AN-BP with HCs found significant differences in diversity and abundances between both subtypes and HCs, as well as some between-subtype differences (Monteleone et al., 2021a; Morita et al., 2015) indicating dysbiosis in both AN-R and AN-BP.

Studies comparing AN microbiota post-nutritional treatment with pre-treatment found increased alpha diversity (Kleiman et al., 2015; Mack et al., 2016; Schulz et al., 2021; Monteleone et al., 2021b; Prochazkova et al., 2021) and improvement in abundance of Roseburia (Mack et al., 2016), Ruminococcus (Kleiman et al., 2015; Mack et al., 2016; Schulz et al., 2021) and Faecalibacterium (Kleiman et al., 2015; Schulz et al., 2021). Nutritional treatment was described as ‘standard’ or ‘strict’, with increased energy, fat and fibre intake, as ‘assisted eating’ (Kleiman et al., 2015; Mack et al., 2016; Monteleone et al., 2021b) or an incremental increase in energy intake was specified (Schulz et al., 2021). Nutritional treatment, in general, reduced dysbiosis seen in pre-treatment AN, with an increase in gut microbiota associated with anti-inflammatory properties such as Roseburia and Faecalibacterium.

Other interesting findings included improved lower GI symptoms with nutrition but no significant remission of upper GI symptoms including bloating and abdominal fullness (Mack et al., 2016) indicating the need for exploring other adjunct treatment to help mitigate these symptoms. Alpha diversity was found to be inversely associated with depression scores (Kleiman et al., 2015; Morkl et al., 2017) and the SCFA butyrate inversely related to anxiety scores (Borgo et al., 2017) indicating a close interaction between the gut microbiome and symptoms suffered from co-morbid psychopathology such as depression and anxiety. Faecal concentrations of neurotransmitters were significantly different in AN, with GABA and dopamine lower than HCs pre-treatment, serotonin lower than HCs post-treatment, indicating an interesting dynamic between these signals pre- and post-treatment (Prochazkova et al., 2021) potentially having a local effect with GI symptoms and visceral sensation but also on inflammation. These could also be potentially related to changes in the gut microbiome with treatment.

Pre-treatment dietary analysis was compared in some studies with energy, carbohydrate (Mack et al., 2016) and fat intakes (Borgo et al., 2017) being significantly lower, while fibre intake being no different (Borgo et al., 2017; Mack et al., 2016) in AN compared with HCs. Diversity was correlated to fibre and Vitamin D intakes (Morkl et al., 2017) indicating the effect nutritional composition and dietary patterns may have on the gut microbiome.

There is some evidence from implementing FMTs from healthy donors into AN recipients with varying results. One study involving an FMT from a related healthy donor to a AN patient with small intestinal bacterial overgrowth and multiple GI symptoms showed significant changes to the microbial composition including increased butyrate-producing such as Roseburia and Faecalibacterium and reduced Prevotella copri at 5–6 months post-FMT. However, microbial composition reverted towards the original state at the 12-month follow up. In addition, there were no reported changes in ED-related and GI symptoms (Prochazkova et al., 2019). Another FMT from an unrelated donor into a AN recipient resulted in significantly improved weight gain (de Clercq et al., 2019). These differing results may indicate multiple factors involved in the maintenance of symptoms in AN.

Some abundances seen pre-treatment in AN including M. smithii and mucin-degrading microbiota may be related to the ability of these microbes to survive the harsh environment of an undernourished gut. Reduction of butyrate-producers such as Roseburia may be related to carbohydrate-poor intake. Moreover, improvement in Roseburia and Faecalibacterium (Kleiman et al., 2015; Mack et al., 2016; Schulz et al., 2021) with nutrition signifies the role undernutrition plays in AN dysbiosis. Lachnospiraceae were correlated with carbohydrate intake (Hanachi et al., 2019) and with shorter duration of treatment (Schulz et al., 2021) again emphasising the role of nutrition. There are commonalities seen in the gut microbiome in some GI disorders as described in Table 4, which may indicate underlying common causative or perpetuating factors including a ‘pro-inflammatory’ gut microbiome. Moreover, changes in the gut microbiome related to depression and anxiety scores may imply their role in GI symptoms and in the gut-brain axis in AN.

Differences seen in the studies in diversity as well as specific microbiota abundances may be explained by heterogeneity of research methods and of cohorts including the location of studies, ages, BMI and duration of illness in participants, dietary patterns prior to study, compensatory behaviours including exercise, as well as factors that generally determine the gut microbiome as listed in Table 1. Other factors that may also influence the gut microbiome are the presence of co-existing mental health disorders such as anxiety and depression as described previously. Thus, gut microbiome presentation in AN may be a result of a number of factors including those related to the history and development of the illness in an individual, co-existing factors as well as factors prior to pathogenesis. It would be useful to explore what influence some of the commonly noted dietary changes in AN have on the gut microbiome.
Table 5
Studies exploring gut microbiome changes in AN.

| Study & Type            | Cohort (n): mean BMI kg/m² | Investigations and time points (T) | Relevant exclusions | Dietary information | Results/outcomes: comparing AN with controls (AN vs control), AN at different time points (for eg: AN T2 vs AN T1), AN subtypes |
|-------------------------|----------------------------|------------------------------------|---------------------|---------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Pfleiderer et al. (2013) | AN (1): 10.4              | Faecal analysis at a single time point before refeeding | Restrictive diet, with vegetables, fruit and dairy | 19 new bacterial species isolated |
| Case study              |                            |                                     |                     |                     | AN vs obese: ↑ M. smithii, ↓ Lactobacillus |
| Armougom et al. (2009)  | AN (9): 12.7 HCs (20): 20.7 | Faecal analysis at 1 time point   | No dietary information | AN vs HCs: Firmicutes, Bacteroidetes, lactobacillus – no difference |
| Cross-sectional study   | 47.1 Obese (20): 27      |                                     |                     |                     | AN vs obese: ↑ M. smithii, ↓ Lactobacillus |
|                         |                            | Faecal analysis at a single time point |                     |                     | AN vs HCs: Firmicutes, Bacteroidetes, lactobacillus – no difference |
| Mörkl et al. (2017)     | AN (18): 15.3 HCs (26): 20.7 | Faecal analysis at 1 time point   | IBD, IBS, use of antibiotics & pre/probiotics within 2 months | AN – treated with a ‘mixed’ diet |
| Cross-sectional study   | Overweight (22): 27 Obese (20): 34.6 | In AN near beginning of inpatient stay |                     |                     | AN vs HCs: Firmicutes, Bacteroidetes, lactobacillus – no difference |
|                         | 22.1 Athletes (20): 22.1 | Depression scores compared         |                     |                     | AN vs HCs: Firmicutes, Bacteroidetes, lactobacillus – no difference |
| Million et al. (2013)   | AN (15): 13.5 HCs (76): 22.4 | Faecal analysis at a single time point from inpatients and outpatients | IBD, use of antibiotics within 6 months | No dietary information |
| Cross-sectional study   | Overweight (38): 27.1 Obese (134): 40 | Depression and anxiety scores compared |                     |                     | AN vs obese: ↑ E.coli |
|                         | 22.1                      |                                     |                     |                     | Obese vs non-obese: ↑ M. smithii, ↑ L. reuteri |
| Morita et al. (2015)    | AN (25): 12.8 HCs (21): 20.5 | Faecal analysis at 1 time point   | IBD, IBS, use of antibiotics & probiotics within 3 months | No dietary information |
| Cross-sectional study   | Age matched HCs (21): 20.5 | Detail of when not described in relation to treatment in AN |                     |                     | AN vs HCs: ↑ total bacterial count, obligate anaerobes, Clostridium coccoidei, C. leptum, Bacteroides fragilis, L. plantarum, Streptococi |
|                         |                            | - AN-R & AN-BP compared           |                     |                     | ↑ acetate & propionic acid faecal levels |
| Borgo et al. (2017)     | AN (15): 13.9 Age matched HCs (15): 22.1 | Faecal analysis at 1 time point   | Dietary intake based of a 3-day food diary | Dietary analysis based of a 3-day food diary |
| Cross-sectional study   |                            | Detail of when not described in relation to treatment in AN |                     |                     | AN vs HCs: ↑ Gram-negative bacteria, Proteobacteria, Enterobacteriaceae, M.smithii |
|                         |                            | Depression and anxiety scores compared |                     |                     | ↑ Firmicutes, Ruminobacteria, Roseburia, Ruminococcus & Clostridia |
|                         |                            | Dietary intake compared            |                     |                     | ↑ Total SCFAs, propionate & butyrate |
|                         |                            |                                     |                     |                     | ↑ Dietary intake in total energy, fats, carbohydrates, but no difference in protein & fibre |
|                         |                            |                                     |                     |                     | ↑ Depression & anxiety scores |
| Hanachi et al. (2019)   | AN (33): 11.7 HCs (22): 21 | Faecal analysis at 1 time point   | Bacteroides uniformis inversely related to BMI | BMI inversely correlated to families |
| Cross-sectional study   | 48-h recall by experienced dietitian | Samples taken within 10 ± 5 days of commencing enteral feeding in AN | AN patients started on a 1 kcal/ml low fibre enteral feed | BMI inversely correlated to families |
|                         |                            | Dietary intake compared            | Known GI pathology, auto-immune disease, use of antibiotics within 2 months | BMI inversely correlated to families |
|                         |                            | ‘Francis score’ compared for functional GI symptoms | Average intake: 1850 kcal/day including < 25 % oral intake | BMI inversely correlated to families |

(continued on next page)
| Study & Type | Cohort (n): mean BMI kg/m² | Investigations and time points (T) | Relevant exclusions | Dietary information | Results/outcomes: comparing AN with controls (AN vs control), AN at different time points (for eg: AN T2 vs AN T1), AN subtypes |
|-------------|-----------------------------|----------------------------------|--------------------|---------------------|---------------------------------------------------------------|
| Monteleone et al. (2021a) | AN-R (17): 15 AN-BP (6): 14.7 HCs (20): 20.3 | Faecal analysis 1 week after standardised diet in AN and in HCs | IBD, malabsorption, coeliac disease, diarrhoea within a month, use of antibiotics within 3 months, probiotics within 2 months | Standardised diets for a week before sampling: AN diet: 1500 kcal/day – 54 % carb, 17 % protein, 29 % fat HC diet: 2000 kcal/day – 45 % carb, 18 % protein, 35 % fat | Verrucomicrobiaceae & Ruminococcaceae - Mean carbohydrate intake correlated to Lachnospiraceae AN-BP vs AN-R: ↑ Actinobacteria, Bifidobacteria, Eubacteriaceae ↓ Odoribacter, Haemophilus AN-R vs HCs: ↓ alpha diversity ↓ Veerrucomicrobia AN-BP vs HCs: Trend towards ↓ alpha diversity AN-BP vs AN-R: ↑ Alpha diversity & Ruminococcus ↓ Alpha diversity inversely related to depression scores AN vs HCs: ↑ Bacteroidetes: Fimicutes at T1, ↓ further at T2, ↓ Firmicutes at T2 ↓ Alpha diversity not different at T1, ↓ at T2 ↑ Mucin-degrading bacteria & M.smithii at T1 ↓ Roseburia at T1, becoming non-significant at T2 ↑ Ruminococcus at T2 ↑ Branched chain fatty acids, markers of protein fermentation, at T1 ↑ Energy & macronutrient intake at T1, but fibre intake comparable ↓ Lower GI symptoms at T2 AN T0 vs HCs: ↓ Alpha diversity ↑ Bacteroidetes: Fimicutes ratio ↑ Actinobacteria, genera Weissella, Coprococcus ↑ Coriobacteriales, Oxalobacteraceae, Parabacteroides AN T1 vs HCs: Alpha diversity not significantly different ↑ Bacteroidetes: Fimicutes ratio ↑ Actinobacteria, Catabacteriaceae, Collinella, Parabacteroides, Catabacter Leuconostocaceae ↑ Trend towards increasing beta diversity with nutrition Faecal metabolomics at specific time points: AN T0: ↓ sugar/ sugar metabolites AN T1: ↓ amino acid and gut microbe derived metabolites HCs: ↓ faecal metabolites of fatty acids and SCFAs At AN T0 - Coprococcus, Clostridium iv, (continued on next page)
| Study & Type | Cohort (n): mean BMI kg/m² | Investigations and time points (T) | Relevant exclusions | Dietary information | Results/outcomes: comparing AN with controls (AN vs control), AN at different time points (for eg: AN T2 vs AN T1), AN subtypes |
|-------------|-----------------------------|-----------------------------------|---------------------|---------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Prochazkova et al. (2021) | AN 1 (52): 14.4<br>AN 2 (52): 17.1<br>HCs (67): 21.9 | - Faecal analysis at 2 time points in AN – admission and discharge. No info on how far into admission or before discharge samples were taken.<br>- faecal analysis for HCs at 1 time point | Diabetes, other chronic disease, severe infections Participants asked to not consume probiotics or aspirin 2 days prior to faecal sampling | No indication of the type of nutritional treatment. Average duration of inpatient stay 51 days in AN. | Core microbiota different in AN compared with HCs. More inter-individual variation in AN compared with HCs. AN1 vs HCs: ↑ Alistipes, Clostridiales, Christensenellaceae, Ruminococcaceae ↓ Faecalibacterium, Agathobacter, Bacteroides, Blautia, Lachnospira ↑ Alpha diversity AN2 vs AN1: ↑ *Megapshaera* ↑ Alpha diversity No significant correlations between EDE-Q, BMI, hyperactivity, disease duration and gut microbiome composition Faecal concentration of neurotransmitters & SCFAs: AN1 vs HCs: ↓ GABA, dopamine, butyrate AN2 vs HCs: ↓ serotonin AN2 vs AN1: ↑ butyrate, ↓ propionate | Roseburia, Termesporobacter, Lachnospiraceae, Ruminococcus negatively associated with EDE score |
| Schulz et al. (2021) | T1 AN (19): % EBW: 75.1<br>T2 AN (19): % EBW: 79.8<br>HCs (20): % EBW: 94.8 | - Faecal analysis at 2 time points for AN: T1- near admission<br>T2- near discharge | GI pathology, coeliac disease, diabetes mellitus, use of antibiotics & probiotics within 4 weeks | AN nutritional treatment: started at 1200 kcal/day increasing in increments of 200 kcal every 2nd day until achieving weight gain of 0.5-1 kg/week | ↑ Anaerostipes AN T2 vs AN T1: ↑ Alpha diversity, Firmicutes, Lachnospiraceae, Ruminococcaceae & Faecalibacterium ↓ Beta diversity significantly different at T1 and remained so at T2 ↑ Abundance of Lachnospiraceae at admission predicted shorter duration of treatment |

BMI: body mass index; EBW: expected body weight; IBD: inflammatory bowel disease; IBS: irritable bowel disease; AN-R: anorexia nervosa, restrictive subtype; AN-BP: anorexia nervosa, binge/purge subtype; HC: healthy control; EDE Q score: Eating Disorder Examination Questionnaire score; GI: gastro intestinal.
6. AN, dietary intake and the gut microbiota

As AN is characterised by change of habitual dietary intake, exploring this may help us interpret typical gut microbiota found in AN. While energy restriction is a fundamental part of AN, studies investigating the nutritional composition of patients’ typical diets have shown a reduction in carbohydrate and fat intakes in restrictive AN, but no significant difference in fibre and protein intake compared to HCs (Mack et al., 2016). It would be useful to examine if this reduction in intake and change in nutritional composition modulates the gut microbiome.

6.1. Energy restriction and effect on the gut microbiota

Murine models have shown a significant change in the gut microbiota with energy restriction (Wang et al., 2018) for example, increases in the abundance of Lactobacillus and Bifidobacterium. In a recent study looking at the gut microbiota in overweight/obese subjects having undergone a very low-calorie diet (VLCD) for 8 weeks, showed an increase in microbes associated with digestion of host-glycans (Akkrmanania) and decrease in species that specialise in digestion of plant polysaccharides (Roseburia, Ruminococcus, Eubacterium) (von Schwartzzenberg et al., 2023). Microbial species tended to revert back to baseline when the VLCD was changed back to a ‘maintenance diet’. A high protein but energy restricted diet in an obese cohort resulted in a similar increase in Akkrmanania and decrease in carbohydrate digestors such as Roseburia (Dong et al., 2020). Increased Akkrmanania species and reduced carbohydrate digestors seen in AN may thus be related to energy restriction, particularly reduction in carbohydrate intake.

Existence of a particular cluster of gut microbiota may increase the host’s susceptibility for weight loss. An interesting study investigating a 40% reduction in energy intake for 3 weeks in 41 subjects with a healthy BMI (mean 23 kg/m²) found that those with a Prevotella predominant enterotype had a significantly higher BMI loss than those with a Bacteroides predominant enterotype (Zou et al., 2020). There was no significant change in enterotype between the baseline and 3 weeks post-intervention.

Similar results have been found in studies with overweight subjects. Prevotella abundance has been associated with a significantly higher weight loss in overweight individuals on a 6-week wholegrain ad-libitum diet compared with those with a Bacteroides abundance (Christensen et al., 2019). A higher fibre diet and a 500 kcal/day energy deficit intervention in overweight subjects, stratified based on their Prevotella/Bacteroides (P/B) ratio, found that those with a high P/B ratio had a significantly higher weight loss than those with a low P/B ratio (Hjorth et al., 2019).

Dietary restriction is one of the factors said to contribute to the establishment of AN (Stice et al., 2010). A monozygotic (MZ) twin discordant study exploring environmental/epigenetic factors by examining the differences between MZ twins affected by AN and co-twins not affected by AN, found that affected twins had a higher likelihood of having started dieting at an earlier age and of GI symptoms than the unaffected co-twins (Thornton et al., 2017). It is conceivable that, in those with a genetic susceptibility to AN and a pre-morbid gut microbiome making them vulnerable to significant weight loss (for example Prevotella predominance), energy restriction and high fibre intake as part of ‘dieting’ could help establish the eating disorder.

Thus, evidence indicates that not only is the gut microbiome influenced by energy restriction and nutritional compositional changes while ‘dieting’, but also that the pre-existence of a particular cluster of gut microbiota may make the host more susceptible to weight loss while dieting.

6.2. Dietary changes and the microbiome in AN

AN often present with dietary changes including becoming a vegetarian or a vegan. A systematic review found correlations between vegetarianism and eating disorders, especially AN (Sergentanis et al., 2020). Here we explore the impact habitual dietary pattern may have on the gut microbiome as well as modulation through changes in these patterns while developing AN.

Habitual dietary intake can have a major impact on the type of gut microbiota in an individual. ‘Westernised diets’ high in protein and fat have been related to the Bacteroides predominant enterotype whereas carbohydrate/fibre rich diets related to vegetarianism/veganism have been associated with the Prevotella predominant enterotype (Glick-Bauer and Yeh, 2014). Examining changes over time within the gut microbiome, there seems to be a resilience within its structure often retaining the core microbes over years (Faith et al., 2013).

Studies investigating vegetarian/vegan diets in the short term and their effects on the gut microbiota have found contradictory results, for example, some showing reduced Bacteroides, increased Prevotella, others showing an increase in both Bacteroides and Prevotella, but many have found a significant shift in microbial composition in the short term (Glick-Bauer and Yeh, 2014). Interestingly, a study based in a Western urban environment comparing long term vegans (at least 6 months) versus omnivores, found only modest differences in the microbiome between the groups (Wu et al., 2016). Another study based in Italy compared intake and the microbiome of vegans, vegetarians (at least 12 months) and omnivores, all within the normal range for BMI. They also did not find significant differences in the vegan microbiota except for an increase in Bacteroidetes compared with omnivores (Losasso et al., 2018). They attributed this to a similar fat intake in all their groups. A recent study found butyrate producing species such as Roseburia hominis, F. prausnitzii and Anaerostipes hadrus associated with dietary intake of unprocessed plant-based foods (Asnicar et al., 2021).

Thus, in AN, it may be a combination of dietary factors including their habitual intake, changes in quantity and composition of their diet and duration of these dietary changes that modulates their gut microbiome. For instance, a patient following a low-fat vegan diet long term may have a very different microbial composition compared with someone with a reduced intake of their regular omnivore diet short-term. It is also interesting that although butyrate producing species seem associated with unprocessed plant foods, a food group that is often eaten in normal quantities by patients with AN, yet the gut microbiome of AN seems to be associated with a low abundance of these bacteria. This may point to other mechanisms in play including the effect of overall energy restriction and inflammatory processes.

6.3. AN, artificial sweeteners and the gut microbiome

Patients with AN are known to regularly use artificial sweeteners (Schebendach et al., 2017) possibly as a non-calorific sweet-tasting reward system. Animal studies have shown a significantly different microbiome and worsened glucose tolerance with intake of sweeteners. A study in healthy weight human volunteers showed a similar change in the gut microbiome and glucose tolerance in those subjects whose gut microbiome was found to be ‘responsive’ to sweetener consumption. Changes seen were an increased Bacteroides: Firmicutes ratio, increase in Bacteroides vulgatus, B. fragilis, decrease in Akkermansia muciniphila & Lactobacillus reuteri. Interestingly, the initial microbial composition of the ‘responders’ was significantly different from the ‘non-responders’ (Suez et al., 2014).

6.4. Influences on the gut microbiome in AN

In summary, the gut microbiome in AN is likely a result of the gut microbial composition prior to onset of illness modulated by the changes in pattern and types of food that the individual follows as part of AN, periods of fasting, whether using exercise, laxatives, vomiting as compensation and biological responses in the gut as well as co-existing conditions such as anxiety and depression. The microbiome by its very nature may influence the type of symptoms that the individual
experiences, for example changes in transit times or production of gases as by-products increasing bloating or inflammatory responses causing symptoms. Nutritional rehabilitation as treatment will modulate the gut microbiome and may eventually support the return of normal gut function including gut microbiome function. However, as nutritional rehabilitation may be associated with symptomatic worsening, it may also be helpful to think of other ways to facilitate normalisation of symptoms and the gut microbiome.

7. Probiotics, prebiotics, their place and rationale for use

From the intense interest in the role of the gut microbiome in host health, it stands to reason that there would be an equal interest in modifying the gut microbiome in promoting health or correcting ‘dysbiosis’ with the introduction of live organisms into the gut or promoting the proliferation of the ‘probiotic’ organisms in the gut. The idea of gut microbial resilience - the tendency of the gut microbiota composition to remain stable or in a state of homeostasis - lends itself to the possibilities of a ‘healthy resilience’ or a ‘dysbiotic resilience’ based on the effects of the microbiota on their host (Coyte et al., 2015; Sommer et al., 2017). The use of probiotics, prebiotics and symbiotics then are attractive propositions for moving the dysbiotic resilience towards a healthy one.

Probiotics are defined as ‘live organisms that, when administered in adequate amounts, confer a health benefit on the host’ (Hill et al., 2014). They may be delivered in foods, for example yoghurts, or as supplements. Currently available probiotics often have microbial strains from the genera *Bifidobacterium*, *Lactobacillus*, *Streptococcus* and the yeast *Saccharomyces*. With probiotics intended for the gut, they need to survive the acidic and alkaline environments while transiting through the various parts of the GI tract, to reach and survive in sufficient numbers at the target site as evidenced by controlled scientific studies.

A prebiotic is defined as ‘a substrate that is selectively utilised by host microorganisms conferring a health benefit’ (Gibson et al., 2017). It not only includes those stimulating proliferation of healthful microbes but also benefits conferred by their products and metabolites on health markers. Commonly studied prebiotics include inulin, fructooligosaccharides (FOS) and galactooligosaccharides (GOS). Although often thought of as synonymous, not all dietary fibres are prebiotics.

7.1. Probiotics and prebiotics in GI disorders

As listed in Table 4, probiotics have been studied in relation to many GI disorders including IBD and functional GI disorders such as FD and IBS. There is some evidence related to probiotic use in coeliac disease.

A systematic review on the use of probiotics in induction and maintenance of remission in IBD found significantly increased remission (RR: 1.74) (Shen et al., 2014). Moreover, maintenance of remission of active ulcerative colitis (UC) with probiotics compared with placebo (RR:1.51), especially related to a multi-strain probiotic VSL.3 (RR: 1.74) (Shen et al., 2014). However, no significant difference was found for Crohn’s disease (Preidis et al., 2020; Shen et al., 2014). Another recent meta-analysis found a trend towards remission with probiotic use, reaching significance when 2 or more probiotic strains were used and when they contained 10^{10}-10^{12} colony forming units. Duration of intervention of 12–16 weeks in UC had a greater effect on disease activity (Zhang et al., 2021). A recent technical review by the American Gastroenterology Association while pointing out the heterogeneity of studies, also found a trend towards improvement in mild/moderate UC with multi-strain probiotic containing *Lactobacillus*, *Bifidobacteria* and *Streptococci* in adults. Although based on fewer studies, there appeared more promise with evidence in UC in children (Preidis et al., 2020).

Proposed mechanisms of action of probiotics in IBD include preventing harmful bacterial adherence to intestinal luminal cells, promoting an ‘anaerobic’ atmosphere therefore preventing Enterobacteria from thriving, promoting an anti-inflammatory effect and improving the intestinal barrier function (Oka and Sartor, 2020).

Overall, indications are that probiotics have the potential to improve GI symptoms in IBD by reducing the pro-inflammatory atmosphere in the gut. The differences in results of the studies may be due to various factors including the starting point of the microbiome and receptiveness to the probiotic, aspects of IBD being different in individuals, their dietary intake/restriction modulating effects and differences in probiotic strains, dosage and duration of intervention.

There are far fewer studies on the effect of probiotics in IBD. A systematic review of 23 RCTs of fibre intake in IBD identified only 6 trials pertaining to interventions fulfilling the criteria for probiotics (Wedlake et al., 2014). The largest study to date investigated the use of inulin-type fructans in treatment of active Crohn’s disease (n = 103), which showed no impact on response or remission rates, indeed there was increased abdominal pain in those receiving the probiotic intervention, although the dose was relatively high (15 g/day) (Benjamin et al., 2011).

Regarding the effects of probiotics in functional GI disorders, there are some indications of benefit with use of a probiotic (*Bacillus* strains or *Lactobacilli* strains) in FD (Drigo et al., 2021; Wauters et al., 2021; Igarashi et al., 2017). A study also showed promising results in infants with cow’s milk protein allergy given a probiotic alongside hydrolysed formula in preventing development of FD symptoms (Nocerino et al., 2019).

Many studies have examined the effects of probiotics in IBS. Recent systematic reviews and meta-analyses have found some trends towards improvement in symptoms (for example, bloating, flatulence, abdominal pain) and symptom scores with multi-strain probiotics (including *Lactobacilli*, *Bifidobacterium* strains and *Streptococci*) (Ford et al., 2018; Sun et al., 2020) although mostly not reaching significance. They also pointed out the heterogeneity among the studies. Another systematic review showed similar trends indicating that a duration of intervention lasting greater than 8 weeks had better results. Interestingly, many studies reported improvement in symptoms with the placebo as well as the intervention, pointing towards support, in general, being helpful (Dale et al., 2019). Promising results were also seen in children with single and multi-strain probiotics in IBS with a duration of intervention > 4 weeks especially in children below age 10 years (Fatahi et al., 2022).

Mechanism of action of probiotics in FGID may include their anti-inflammatory effect, their modulation of visceral hypersensitivity, their effect on gut transit and their effect on mood and anxiety. Effect on GI symptoms may in turn have an effect on their well-being and quality of life, reducing the effects of stress and anxiety further. Differing results may be due to heterogeneity of cohorts and probiotic administration.

With the use of prebiotics in IBS and functional bowel disease, a systematic review did not find a significant difference in symptomatic relief when comparing probiotics with placebo. The intervention was however associated with an increase in *Bifidobacteria* abundance, strains of which have been associated with mood improvement. Despite this, there was no significant difference in anxiety and depression scores. (Wilson et al., 2019). It is useful to note that a low ‘FODMAP’ (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) diet which is low in natural prebiotics has substantial evidence in reducing symptoms in IBS (Whelan and Staudacher, 2022).

A review looking at the use of probiotics, *Bifidobacteria* or *Lactobacilli*, in coeliac disease found inconsistent results, with some positive associations with reduction in GI symptoms, changed gut microbiota and improved immune profile, while others found no difference (Akbeng et al., 2020).

7.2. Probiotics and prebiotics in mental health conditions

A systematic review and meta-analysis on the effects of probiotics and prebiotics in depression and anxiety found significant effects of probiotics compared with placebo in depression and anxiety (Liu et al., 2019). In depression, the effect was larger with samples in the clinical/medical settings compared with those in the community. Use of
probiotics for 4 weeks or more and multi-strain probiotics were found to be more effective. With anxiety as an outcome measure, there was a modest effect of the use of probiotics. As all the included samples were from the community, this review could not comment on clinical anxiety. In contrast, prebiotics did not have a significant effect on depression or anxiety (Liu et al., 2019). The mechanisms proposed by which probiotics affect anxiety and depression include their effect on the vagus nerve and afferent signals via microbial molecules (Dalton et al., 2019), reducing inflammatory response and the modulation of GABA and 5HT signalling (Foster and McVey Neufeld, 2013).

7.3. Probiotics in AN

Currently there is only preliminary evidence on the use of probiotics in AN. An early study compared the use of milk with probiotic yoghurt containing Lactobacillus. bulgaris & Streptococcus. thermophilus in 22 malnourished children (70–80 % weight for height) and 12 controls (100 % weight for height) in Morocco, while also examining the effect of these interventions in 27 adolescent females (mean BMI 15.5 kg/m²) with AN. These AN patients received either yoghurt or milk for 10 weeks followed by a crossover period for 10 weeks of the opposite intervention alongside standard refeeding. While malnourished children in both the milk and yoghurt groups and controls having yoghurt had significant increases in γ interferon, in AN, probiotic yoghurt was associated with a significantly higher increase in γ interferon compared with milk (Solis et al., 2002) indicating an effect of the probiotic on immune modulation. Another study with 30 AN patients and 35 controls, both groups randomised to having either probiotic yoghurt (L. bulgaris & S. thermophilus containing) or milk for 10 weeks showed an increased γ interferon and an increased CD4:CD8 ratio in the yoghurt group (Nova et al., 2006). Such immune modulations may also have a positive effect on the GI tract potentially reducing GI symptoms in AN.

Results are awaited from a study comparing the use of a multi-strain probiotic (Lactobacilli & Bifidobacteria strains) with a placebo for 6 months alongside treatment as usual in 60 adolescents with AN and 60 HCs (Gröbner et al., 2022). Outcomes examined will be changes in weight, ED psychopathology, GI symptoms and the gut microbiome over 12 months. This study should add crucial evidence about the suitability of this adjunct treatment as well as its effects on GI symptoms.
GI symptoms in AN seem to mainly resolve with standard nutritional rehabilitation (West et al., 2021). However, increasing GI symptoms during the course of treatment and persistence of symptoms beyond nutritional recovery are also seen (Boyd et al., 2010; Chapelon et al., 2021; Mack et al., 2016). There is evidence of gut dysbiosis in AN with some similarities of microbiota seen in AN and IBD. Both conditions are associated with aberrant immune responses. Initial studies in AN with probiotics have shown improvement in immune responses (Nova et al., 2006; Solis et al., 2002). Probiotics have been used in IBD, FGID and in mental health illnesses such as depression and anxiety with some indication of improvement in symptoms. Therefore, the use of probiotics in AN should theoretically be a helpful adjunct to nutritional rehabilitation with the potential to mitigate GI symptoms and improve immune responses. However, the use of prebiotics for amelioration of GI symptoms has scant evidence so far.

Multi-strain probiotics with Lactobacilli and Bifidobacteria seem to have the most evidence with IBD and IBS as well as in depression and anxiety. Bacterial strains that are in direct competition with each other appear to have a stabilising effect in models, attributed to reducing excessive positive feedback loops, in turn preventing one microbe dominating (Coyte et al., 2015). It is possible that a similar mechanism is in play when multi-strain probiotics from similar genera are used in vivo helping establishment of these microbes in the gut. Treatment duration greater than 4 weeks seems to be most effective with IBD, depression and anxiety. Preliminary studies of probiotic use in AN have shown some immune modulatory effects with a 10-week intervention (Nova et al., 2006; Solis et al., 2002). Durations of treatment were much longer with UC mainly used for maintenance of remission. It may be that effects on anxiety and low mood occur earlier but changing dysbiosis and the immune system response require longer treatment. As both anxiety related GI symptoms and functional and immunological GI symptoms may be a part of AN, it seems feasible that probiotics, shorter and longer term, may be helpful in reduction of symptoms.

There exists an argument that experiencing GI discomfort during recovery in AN is of therapeutic value, perhaps as a way of modulating the gut-brain axis feedback and fear de-conditioning. While it is important for AN sufferers to acknowledge and accept appetite and satiety cues as being normal and necessary (Treasure and Alexander, 2013), our premise of supporting them manage their GI symptoms should not be a counter-argument. Evidence from gut microbiome research so far indicates its potential role in magnifying GI symptoms suffered and so support with ameliorating these is in keeping with ‘normalising’ appetite and satiety cues. Moreover, evidence also indicates that gut microbiome changes have a role beyond their effect on GI symptoms, including modifying signalling through the gut-brain axis and immune-modulation making further exploratory research in this field all the more important.

8.1. Limitations in the literature and directions for future research

The few studies so far in AN have been on the impact of probiotics on the immune system. While the gut microbiome as a target for treatment is a theoretical possibility, more research is needed in being able to recommend this as adjunct to current treatment. The planned study by Gröbner et al. (2022) is a starting point in examining the use of probiotics in AN and GI symptom recovery. Fig. 1 includes our thoughts on research avenues exploring gut microbiome and metabolomics in AN and the potential for initiating change in the microbiome towards health.

While there are indications that standard nutritional treatment in AN has positive effects on the gut microbiome and on GI symptoms, it was beyond the scope of this article to explore the particulars of nutritional treatment including nutritional composition that may be beneficial towards the microbiome and GI symptoms. It would be important to examine these further to pinpoint nuances in nutritional rehabilitation that may enhance the recovery process and also mitigate GI symptoms.

9. Conclusions

AN appears to have similarities to other GI disorders and to mental health disorders where the mechanism of action of gut microbiota has been postulated in relation to pathology and gut symptoms and the use of probiotics have been shown to have some effect. It is therefore possible that the use of probiotics in AN may be a helpful adjunct to current treatment. However more studies are needed to prove efficacy.
ConFLICT OF INTEREST

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