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An open-label pilot trial of faecal microbiome transfer to restore the gut microbiome in anorexia nervosa: protocol

Brooke C Wilson [1], José G B Derraik [1,2], Benjamin B Albert [1], Karen S W Leong [1], Ry Y Tweedie-Cullen [1], Christine Creagh,[1] Marysia Depczynski,[1] Taygen Edwards [1], Tommi Vatanen [1,3], Hiran Thabrew [4,5], Wayne S Cutfield [1], Justin M O'Sullivan [1]

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For numbered affiliations see end of article.

Correspondence to
Justin M O'Sullivan;
justin.osullivan@auckland.ac.nz

ABSTRACT

Introduction Individuals with anorexia nervosa (AN) harbour distinct gut microbiomes compared with healthy individuals, which are sufficient to induce weight loss and anxiety-like behaviours when transplanted into germ-free mice. We hypothesise that faecal microbiome transfer (FMT) from healthy donors would help restore the gut microbiome of individuals with AN, which in turn, may aid patient recovery.

Methods We aim to conduct an open-label pilot study in 20 females aged 16–32 years in Auckland, New Zealand who meet the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) criteria for AN and have a body mass index 13–19 kg/m^2. We will recruit four healthy, lean, female donors, aged 18–32 years, who will undergo extensive clinical screening prior to stool donation. Faecal microbiota will be harvested from donors and double encapsulated in delayed release, acid-resistant capsules. All participants will receive a single course of 20 FMT capsules (five from each donor) which they can choose to take over two or four consecutive days. Stool and blood samples will be collected from participants over a period of 3 months to assess their gut microbiome profile, metabolome, levels of intestinal inflammation and nutritional status. Our primary outcome is a shift in the gut microbiome composition at 3 weeks post-FMT (Bray-Curtis dissimilarity). We will also monitor participants' body composition (whole-body dual-energy X-ray absorptiometry scans), eating disorder psychopathology, mental health and assess their views on, and tolerability of, treatment. All adverse events will be recorded and reviewed by an independent data monitoring committee.

Ethics and dissemination Ethics approval was provided by the Central Health and Disability Ethics Committee (Ministry of Health, New Zealand, 21/CEN/212). Results will be published in peer-reviewed journals and presented to both scientific and consumer group audiences.

Trial registration number ACTRN12621001504808.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This pilot trial will investigate faecal microbiome transfer (FMT) as a therapy for gut microbiome restoration in young women with anorexia nervosa (AN).
- ⇒ This study has been codesigned in consultation with eating disorder specialists and recovered individuals to minimise participant harm and stress.
- ⇒ The use of high-resolution shotgun metagenomic sequencing will allow for a comprehensive gut microbiome assessment and longitudinal tracking of donor strain engraftment.
- ⇒ While we will monitor clinical features of AN throughout the trial, efficacy of FMT on clinical outcomes cannot be assessed due to its design without a control group.
- ⇒ By focusing on young women with AN, study findings may not necessarily apply to the broader population of individuals with AN.

INTRODUCTION

Anorexia nervosa (AN) is a complex and debilitating eating disorder characterised by extremely restrictive eating behaviour, very low body weight, a fear of weight gain and body image distortion.[1] AN has the highest mortality rate among psychiatric disorders (standardised mortality ratio of 5.86[2]) and is often accompanied by comorbidities including anxiety, depression, autoimmune disorders and functional gastrointestinal disorders.[2–5] AN usually begins in adolescence and is more common in women (lifetime prevalence of 1.4% compared with 0.2% in men[6]). While its exact aetiology is unclear, the development of AN likely stems from both environmental triggers[7] and genetic predisposition.[8 9] In addition, emerging evidence suggests that the gut microbiome might also be involved in AN as an important regulator of appetite, mood and metabolism.[10–13]

Multiple studies have shown that the gut microbiome is perturbed in individuals with AN.[14–25] Early reports suggested that AN microbiomes were typically less diverse when compared against healthy age-matched controls.[19 21] However, more recent observations do not support a simple reduction in microbial diversity being linked to AN,[16 17 24] but rather, a difference in the relative abundances of specific taxa. In particular, a recent systematic review determined that AN individuals harboured proportionally less fiber-utilising taxa (eg, *Roseburia* sp.) and more mucin-degrading taxa (eg, *Akkermansia* sp. and *Methanobrevibacter smithii*).[26] Degradation of the intestinal mucus lining can lead to increased permeability and translocation of bacterial products into circulation,[27 28] both of which have been observed in AN.[29–32]

While the gut microbiome alterations observed in AN are likely a consequence of severe caloric restriction and psychological stress, the gut microbiome itself has also been suggested to play a role in perpetuating symptoms of the disorder.[33] For example, individuals with AN have higher blood levels of caseinolytic-protease-B (ClpB), a protein produced by commensal gut species.[32] ClpB shares homology with the human anorexigenic α-melanocyte-stimulating hormone and may therefore mimic its function to suppress appetite and increase energy expenditure.[34]

A contributory role of the gut microbiome in AN symptomatology was demonstrated when germ-free mice were inoculated with the gut microbiome derived from either healthy human donors or donors with AN.[35] The offspring of mice who received the 'AN microbiome' showed reduced body weight and a concomitant reduction in food intake. Interestingly, when the 'AN microbiome' mice ate the same amount of food as the 'healthy microbiome' mice, they gained less weight, suggesting that they were less efficient at converting food into body mass.[35] Furthermore, the 'AN microbiome' mice had reduced serotonin levels and displayed anxiety-related and compulsive behaviours.[35] Similar weight gain differences were observed in another germ-free mice experiment that utilised both AN and healthy control donors, with differences being linked to altered expression of appetite-suppression and thermogenesis genes.[17] By contrast, another study found no difference in body weight or daily food intake between mice receiving transplantations of AN-donor or healthy-donor microbiota.[36] Further research is therefore required to determine whether the gut microbiome acts as a potential mediator of disease in AN and is thus a suitable target for therapeutic intervention.

Current treatment approaches for AN are multidisciplinary and focus on nutritional rehabilitation, weight restoration and cognitive behavioural therapy.[37] However, despite these efforts, approximately 40% of individuals will only partially recover from the disorder, with a further 20% maintaining a chronic course of illness over their lifetime.[38] Even after nutritional interventions and weight restoration, the gut microbiome in individuals with AN remains distinct from that of healthy individuals,[19 20] potentially contributing to relapse of symptoms.[33] Therefore, strategies designed to restore the gut microbiome in AN may be of clinical benefit when used in conjunction with current nutritional rehabilitation therapies, and warrant further investigation.

Faecal microbiome transfer (FMT) involves the transfer of gut microbiota from healthy donors to recipients with gut dysbiosis. This therapy has proven highly effective for treating recurrent *Clostridioides difficile* infections, and can rapidly restore the diversity and functions of the gut microbiome in these patients.[39] FMT has also been trialled in other disorders associated with less severe forms of gut dysbiosis, such as obesity,[40–42] metabolic syndrome,[43–46] inflammatory bowel disease,[47] irritable bowel syndrome[48] and autism.[49] While FMT cannot cure these multifaceted conditions, its ability to alter the gut microbiome has led to various therapeutic benefits among recipients including improvements in fat distribution,[42] metabolic syndrome,[42] insulin sensitivity,[43 44] intestinal permeability,[50] gut inflammation,[51] gastrointestinal symptoms[52] and social behaviours.[49]

Given the role of the gut microbiome in regulating appetite, mood and metabolism,[10–12] restoring the gut microbiome in individuals with AN may act as a stepping stone towards improved patient recovery. There have been two published case reports of FMT in patients with AN.[53 54] In both instances, the patients showed an increase in gut microbiome diversity following FMT, however, metabolic improvements and weight restoration were only observed in one case.[53] Further research is therefore necessary to better understand whether FMT represents a viable treatment option for individuals with AN.

The aim of this pilot study is to assess the feasibility of using FMT to help restore the gut microbiome in individuals with AN. Rather than using invasive FMT administration approaches with limited scalability, our study will employ validated methods for donor microbiome encapsulation.[55 56 57] To help boost microbiome diversity, participants will receive an equal number of capsules from four donors who will be selected after extensive health and microbiome screening. Participants will be monitored for adverse events, have their gut microbiome profiled and be clinically assessed for up to 3 months post-FMT.

METHODS

Study design

This study is a one-arm, open-label pilot trial investigating the safety, tolerability and potential of FMT to restore the gut microbiome in young females with AN. The study will be conducted at the Liggins Institute's Clinical Research Unit (University of Auckland), in Auckland, New Zealand.

Participants

We aim to recruit 20 female participants aged 16–32 years who meet the DSM-5 criteria for AN and have a body mass index (BMI) 13–19 kg/m^2 (table 1). Participants will be recruited through engagement with local eating disorder clinics, the Eating Disorders Association of New Zealand

Table 1 Eligibility criteria for participants and donors

Participants	Donors
Inclusion	
Biological female at birth	Biological female at birth
16–32 years of age	18–32 years of age
BMI 13–19 kg/m^2	BMI 18.5–25 kg/m^2
Formal diagnosis of AN by an eating disorder specialist*	Total body fat ≤33% (as assessed by whole-body DXA)
Medically stable†	Healthy diet (≥4 portions of fruit and/or vegetables/day)
Able and willing to swallow the treatment capsules	Regular exercise (moderate-to-vigorous physical activity for ≥3.5 hours/week)
Able and willing to comply with the clinical assessments	Regular bowel habit (passing stools at least once every 2 days)
Exclusion	
Known allergies to any foods or medications	Positive screening test for any transmissible pathogen or multidrug resistant organism listed in table 2
Use of antibiotics or probiotics in the month prior to treatment	Gastrointestinal disease (eg, inflammatory bowel disease, irritable bowel syndrome, coeliac disease or eosinophilic oesophagitis)
Regular oral steroid treatment or daily application of potent topical steroids extensively to the body	Metabolic disorder (eg, diabetes, metabolic syndrome, hypertension, dyslipidaemia or dysglycaemia)
Compromised immune system	Impaired fasting glucose (>5.9 mmol/L) or elevated HbA1c (>41 mmol/mol)
Any other chronic illness affecting gut or metabolic health	Asthma or eczema requiring regular prophylaxis or treatment
Thoughts of self-harm and/or suicidal ideation‡	Autism spectrum disorder
Current or planned pregnancy during the course of the study	Previous diagnosis of mental health issues including eating disorders
Regular laxative use	Current or history of malignancy
	Use of oral antibiotics or probiotic supplements in the past 3 months
	Regular binge drinking (>5 standard alcoholic units per session at least once a week)
	Past or present use of recreational drugs, tobacco or vaping
	Current or past pregnancy
	Overseas travel in the past 2 weeks§

*Meeting the DSM-5 criteria, 307.1.
†In accordance with Starship Children's Hospital's clinical guidelines[70] (ie, resting heart rate >50 bpm; no postural drop in blood pressure or rise in heart rate, no electrolyte abnormalities, and temperature >35°C and <37.8°C).
‡Based on response to question 9 of the Patient Health Questionnaire-9 (PHQ-9).[60]
§Donors who have travelled overseas will need to wait a minimum period of 2 weeks from their arrival back in New Zealand before donating.
AN, anorexia nervosa; BMI, body mass index; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, fifth edition; DXA, dual-energy X-ray absorptiometry.

(EDANZ) and social media. Study brochures and a detailed participant information sheet will be supplied to potential participants and their caregivers who are interested and considered eligible by their specialist physician. Participants will have the opportunity to ask any questions about the study before they decide to consent. Participants will be able to withdraw from the study at any time.

Donors

To minimise treatment heterogeneity, we will attempt to use the same four FMT donors throughout the trial. We aim to recruit up to eight female stool donors to ensure that we have sufficient reserve donors if one becomes unwell, unavailable or otherwise ineligible during the study. Donors will be recruited through the University of Auckland's internal email list and via social media advertising. Potential donors will be interviewed over the phone to assess general eligibility criteria (ie, health and lifestyle parameters) before being invited to our clinic for further screening. We will employ the same donor screening protocol used in our previous FMT trial[57] (table 1). Donors will be screened to ensure the absence of disease and any transmissible viral, bacterial or protozoal pathogens (table 2). Donors will be given a detailed participant information sheet to read, and will have the opportunity to ask any questions about the study before they decide to consent. Donors will be able to withdraw from the study at any time. Any capsules produced prior to withdrawal may be kept for use in the study.

Table 2 Pathogen screening for donors

	Bacteria	Parasites	Viruses
Blood	*Treponema pallidum* (syphilis)	*Strongyloides* spp.*	Hepatitis A, B, C HIV
Stool	*Campylobacter* spp. *Clostridioides difficile* toxin A/B Diarrheagenic *Escherichia coli/Shigella* spp.: ► Enteroaggregative *E. coli* (EAEC) ► Enteroinvasive *E. coli* (EIEC) ► Enteropathogenic *E. coli* (EPEC) ► Enterotoxigenic *E. coli* (ETEC) ► Shiga-like toxin-producing *E. coli* (STEC) *Helicobacter pylori* Multidrug-resistant organisms: ► Carbapenem-resistant organisms ► Extended-spectrum beta-lactamase-producing Enterobacteriaceae ► Vancomycin-resistant *Enterococcus* spp. *Plesiomonas shigelloides* *Salmonella* spp. *Vibrio* spp. *Yersinia enterocolitica*	*Cryptosporidium* spp. *Cyclospora cayetanensis* *Entamoeba histolytica* *Giardia lamblia* Any ova, cysts, or parasites upon microscopic examination	Human adenovirus F 40/41 Astrovirus spp. Norovirus GI/GII Rotavirus A Sapovirus spp.
Nasal			SARS-CoV-2

*Only performed if the donor has a history of travel to the tropics.

Treatment

All participants will receive the same treatment. We will use a multidonor FMT approach in which recipients receive 20 capsules containing the gut microbiota from four healthy female donors (five capsules from each donor). The total FMT dose corresponds to 10 g of concentrated gut microbiota (2.5 g/donor).

FMT capsule preparation

Donors will be asked to visit the Clinical Research Unit every 6 months to provide fresh stool samples for capsule production. Donor screening will be repeated for every capsule batch (tables 1 and 2). If multiple stool samples from the same donor are required for one batch of capsules, repeat screening will only be performed if it has been >2 weeks since their last screen. If any of the four selected donors fail their repeat screening, we will contact and invite one of the reserve donors for rescreening.

We will use validated methods for gut microbiome encapsulation as described previously.[57] Donor stools will be processed individually for encapsulation under aerobic conditions. Immediately after donation, stool will be blended with 1:2 volumes of 0.9% saline solution and sieved to remove particulate matter. To remove any remaining particulate matter, the faecal slurry will be centrifuged (200× gravity, 5 min, room temperature). The resulting supernatant will be decanted into a fresh tube and centrifuged (5000× gravity, 15 min, room temperature) to concentrate the bacterial pellet. The bacterial pellet will be resuspended at a concentration of 1 g/mL in a cryoprotective solution (15% glycerol, 0.9% saline) and 500 µL aliquots will be dispensed into size 0 delayed release capsules (DRcaps, Capsugel, Sydney, Australia). The size 0 capsules will be closed and secondarily sealed in size 00 DRcaps capsules. DRcaps are specifically designed to mask taste and odour, resist stomach acid and deliver their contents to the proximal bowel.[58] Capsules will be stored at −80°C for up to 6 months.

FMT capsule administration

Treatment appointments will be scheduled for early morning and the participant will need to have fasted overnight for at least 8 hours. Given the high rates of laxative abuse in individuals with AN,[59] we will not be performing a laxative bowel cleanse prior to FMT. Treatment will be spread over 2 days (10 capsules/day) or 4 days (5 capsules/day) depending on the participant's preference. Capsules will be swallowed with water under direct supervision from a research nurse or clinician. Participants will be asked to postpone their breakfast until 1 hour after swallowing the capsules to help minimise the length of time the capsules will spend in the stomach.

Study schedule

Participants will have an enrolment appointment where we will explain the study in detail, assess their eligibility and obtain their written informed consent. During this visit, we will perform a whole-body dual-energy X-ray absorptiometry (DXA) scan and administer the Patient Health Questionnaire 9 (PHQ-9)[60] to confirm they meet the BMI criteria and do not have feelings of self-harm/suicidal ideation. We will also collect a stool sample from the participant during this visit and schedule their baseline assessment for 3 weeks time (table 3). The first treatment dose will be given at baseline after all assessments have been completed. Subsequent treatment doses will be

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Table 3 Study schedule

Scheduling	Screening and enrolment	Baseline and treatment day 1	Treatment day 2*	48 hours	1 week	3 weeks	6 weeks	12 weeks
	3 weeks before baseline	3 weeks after enrolment	1 day after baseline	2 days after baseline	1 week after baseline	3 weeks after baseline	6 weeks after baseline	12 weeks after baseline
Visit type	Clinic	Clinic	Clinic	Phone call	Phone call	Phone call	Clinic	Clinic
Eligibility screen	✓							
Informed consent	✓							
FMT treatment		✓	✓					
Adverse events			✓	✓	✓	✓	✓	✓
Tolerability questionnaire			✓					
Background questionnaire		✓						
Eating disorder symptoms (EDEQ)[66]		✓					✓	✓
Depression symptoms (PHQ-9)[60]	✓	✓					✓	✓
Anxiety symptoms (GAD-7)[67]		✓					✓	✓
Stool sample	✓	✓				✓	✓	✓
Blood sample		✓					✓	✓
Whole-body composition scan (DXA)	✓							✓

*Study timeline is based on a 2 day treatment schedule. If a participant decides to spread their treatment over four consecutive days, the tolerability questionnaire will be administered on the final day of treatment (day 4) and the 48 hour phone call will be 24 hours after the final dose of capsules.
DXA, dual-energy X-ray absorptiometry; EDEQ, Eating Disorder Examination Questionnaire; FMT, faecal microbiome transfer; GAD-7, General Anxiety Disorder 7-item scale; PHQ-9, Patient Health Questionnaire 9.

scheduled the following consecutive day/s depending on the participant's preference for a 2 day or 4 day treatment schedule. Follow-up clinical assessments will be scheduled for 6 and 12 weeks after baseline. Participants will also be asked to collect a stool sample at home 3 weeks after their baseline assessment.

Data collection and follow-up

Stool sample collection

Stool samples will be collected from participants 3 weeks before treatment, at baseline and 3, 6 and 12 weeks after treatment to assess changes in the gut microbiome, metabolome and levels of intestinal inflammation. Where possible, stool samples will be collected on site except for the '3 weeks before' and '3 weeks after' treatment samples which the participant will collect at home. If a participant cannot produce a stool sample during their clinical assessment visit, they will be given a stool collection kit to take home. Collection kits contain a stool catcher, disposable gloves, specimen bag, specimen pottle, DNA/RNA Shield Faecal Collection tube (#R1101, Zymo Research, Irvine, California, USA), and a step-by-step instruction card. If the sample is collected at home, participants will be asked to store their samples within their home freezer until their next appointment or arrange for collection by a member of the research team. On receipt, stool samples will be aliquoted and stored in −80°C freezers at Te Ira Kāwai—Auckland Regional Biobank. Stool collected in the DNA/RNA Shield Faecal Collection tube will be reserved for gut microbiome assessment. Stool collected in the specimen pottle (ie, not containing any stabilisation buffer) will be reserved for metabolomics and intestinal inflammation assays (eg, calprotectin, lactoferrin and S100A12[61]).

Gut microbiome profiling

DNA and RNA will be extracted using the ZymoBIOMICS MagBead DNA/RNA kit (Zymo Research, #R2136) according to the manufacturer's instructions with the addition of a bead bashing lysis step (Zymo Research, #S6002-96-3). Shotgun metagenomic and metatranscriptomic sequencing will be performed by a commercial provider using Illumina's paired-end sequencing technology. Sequencing data will be processed as performed previously,[56] using bioBakery tools for meta'omic profiling.[62] In particular, StrainPhlAn[63] will be used to generate single nucleotide polymorphism (SNP) haplotypes representing the dominant strain of any given species within a sample. We will use these SNP haplotypes to compare the genetic similarity of donor and recipient strains before and after treatment to assess the proportion and stability of donor strain engraftment.

Table 4 Blood test schedule for all clinical assessments (baseline, week 6 and week 12)

	Safety monitoring	Study outcomes
Electrolytes (sodium, potassium)	✓	✓
Creatinine	✓	✓
Ferritin	✓	✓
Total protein	✓	✓
Albumin	✓	✓
Alkaline phosphatase	✓	✓
Alanine aminotransferase	✓	✓
Gamma-glutamyl transferase	✓	✓
Aspartate aminotransferase		✓
C-reactive protein		✓
Cholinesterase		✓
Folate		✓
Vitamin B_{12}		✓
Cortisol		✓
Free thyroxine		✓
Thyroid stimulating hormone		✓
Serotonin		✓

Blood sample collection

Blood samples will be collected at baseline, 6 weeks and 12 weeks after treatment to assess nutritional status, inflammation and liver/thyroid function (table 4). A subset of these tests will be performed in real-time throughout the study period for safety monitoring. These tests will also be repeated at the completion of study for evaluation of study outcomes, avoiding any potential batch effects.

Anthropometry and body composition

Anthropometric measurements and body composition assessments are potentially triggering for people with AN. Discussions and feedback from eating disorder specialists and recovered individuals have confirmed that regular body weight measurements (specifically standing on scales) throughout the study could cause unnecessary stress for participants given the primary focus of the study is on gut microbiome restoration. However, these assessments are important for safety monitoring and detection of potential adverse events. Therefore, a whole-body DXA scan will be performed at enrolment and 12 weeks after treatment to assess body weight and composition (including the proportion of lean mass and fat mass, and bone mineral density). We will also measure the participant's height barefoot using a wall-mounted stadiometer and combine this information with the DXA-generated body weights to calculate BMI.

Questionnaires

All questionnaires will be completed by the participants online using data capture tools from the web-based research platform, REDCap (Research Electronic Data Capture).[64] At the beginning of the study, we will collect background demographic information from participants including their age, sex assigned at birth, gender identity, self-reported ethnicity, socioeconomic status (based on physical address), age when first diagnosed with AN and current medications. Socioeconomic status will be estimated using the New Zealand Indices of Multiple Deprivation.[65]

After their final dose of capsules, participants will complete a short questionnaire to gather their views and experience of taking the treatment. Specifically, participants will be asked how difficult it was to swallow the capsules, whether they experienced any unpleasant side effects during and/or after swallowing the capsules and whether they would consider taking the treatment again if it was later shown to be beneficial for recovery.

Participants will also complete three established health questionnaires at baseline, 6 weeks and 12 weeks after treatment: (1) Eating Disorder Examination Questionnaire (EDEQ V.6.0),[66] (2) Patient Health Questionnaire 9 (PHQ-9) for symptoms of depression[60] and (3) General Anxiety Disorder 7-item scale (GAD-7).[67]

Safety monitoring

By adopting strict selection criteria for donors, we will reduce the risk of infection via FMT by minimising the potential transmission of pathogenic organisms. Participants will take each dose of FMT in our clinic under the supervision of a research clinician and/or nurse, where they will remain under close monitoring for at least 1 hour afterwards. Based on our previous experience and existing evidence, it is unlikely that participants will experience any severe adverse events.[42] However, participants will be instructed to seek immediate medical attention if they develop any severe adverse reactions following treatment. We will contact participants 24 hours after ingestion of each set of capsules, as well as 1, 3, 6 and 12 weeks after treatment to enquire about any adverse side effects. Specifically, participants will be asked to report on the following events: loose or bloody stools, abdominal pain, vomiting, nausea, constipation, flatulence, bloating, fever, malodorous burps, flu-like symptoms, allergic symptoms, appetite, fatigue and agitation. Adverse events will be graded in accordance with the Common Terminology Criteria for Adverse Events V.4.0 (CTCAE).[68]

In addition, we will monitor blood markers of nutritional status and liver function (table 4), any available body weight records as provided by the participant's clinical care team, and questionnaire scores throughout the study in case any of the participants' health starts deteriorating. If the participant answers 'several days', 'more than half the days' or 'nearly every day' to PHQ-9, question 9 'Thoughts that you would be better off dead or of hurting yourself in some way', the research clinician will interview the participant further and provide them with safety management information to take home. Before the participant leaves the clinic, the research clinician will also recommend clinical follow-up and contact the participant's routine care provider and/or the research psychiatrist to ensure additional mental health support is provided.

These clinical and adverse event data will be reviewed by an independent data monitoring committee (DMC) who can decide to stop the trial if the safety of participants is thought to have been compromised. Any serious adverse event or clinical result will be notified immediately to the DMC.

We will strongly advise participants to bring a support person to their study appointments. The support person could be a family member, friend or member of their support team. Following discussions with our advisers, this person would support the participant during and after the clinic visits, provide comfort and reassurance to the participant throughout the study, and act as an additional point of contact in case the participant becomes uncontactable during the study period.

Outcomes

Primary outcome

- A shift in gut microbiome composition at 3 weeks post-FMT (Bray-Curtis dissimilarity). The shift should exceed the drift in gut microbiome composition measured over the 3 weeks between enrolment and baseline.

Secondary outcomes

- Adverse events associated with FMT treatment.
- Proportion of participants who swallow all 20 treatment capsules.
- Proportion of participants who would consider having the treatment again if effective.
- Gut microbiome diversity, composition and functional potential at 3, 6, and 12 weeks post-FMT.
- Donor strain engraftment at 3, 6 and 12 weeks post-FMT.
- Intestinal inflammation at 3, 6 and 12 weeks post-FMT.
- Blood markers of nutritional status and liver/thyroid function at 6 and 12 weeks post-FMT.
- Eating disorder symptoms at 6 and 12 weeks post-FMT.
- Depression symptoms at 6 and 12 weeks post-FMT.
- Anxiety symptoms at 6 and 12 weeks post-FMT.
- BMI at 12 weeks post-FMT.
- Body composition at 12 weeks post-FMT.

Sample size calculation

Our primary outcome is a shift in the gut microbiome composition at 3 weeks post-FMT. Because we do not have a control group to compare against, we will instead collect a stool sample 3 weeks prior to FMT to assess the background drift in the gut microbiome over a 3 week period without any intervention. We will use the Bray-Curtis dissimilarity index to compare gut microbiome composition profiles between sampling time points and test for a difference in these values using a paired t-test. To identify a shift in the gut microbiome above background drift, we will need 18 participants (80% power, alpha=0.05). This calculation was based on data from our previous FMT trial[42] comparing the gut microbiome shifts between 39 FMT and 44 placebo recipients over 6 weeks (Bray-Curtis dissimilarity to baseline; FMT mean 0.574, Placebo mean 0.416, delta=0.158, SD=0.163, t-test $p<0.001$). To account for a potential dropout rate of 10%, we aim to recruit at least 20 participants who complete treatment and the primary outcome assessment.

Statistical analyses

We will perform both intention-to-treat and per-protocol analyses. Per-protocol analyses will only include data from those who complete the full treatment dose. Baseline demographics and clinical characteristics will be summarised using descriptive statistics. Gut microbiome shifts will be assessed by calculating the Bray-Curtis dissimilarity index to and from baseline using species-level relative abundance profiles. To assess the primary outcome, a two-sided paired t-test will compare the potential shift in gut microbiome composition 3 weeks before treatment to the shift 3 weeks after treatment. No imputation will be performed for missing data, and statistical significance will be set at $p<0.05$.

Multivariate Association with Linear Models (MaAsLin2) will be used to examine changes in the relative abundances of microbial taxa and their encoded functions in response to treatment. We will also use MaAsLin2 to explore associations between microbiome features and clinical outcomes.

Changes in clinical outcomes from baseline will be assessed using paired t-tests (parametric) or Wilcoxon signed rank tests (non-parametric), as appropriate. However, we acknowledge that we cannot make any treatment efficacy claims based on these paired within-group analyses and without a control group.

Patient and public involvement

This study has been codesigned in consultation with members from the Eating Disorders Association of New Zealand (EDANZ) as well as women who have previously recovered from AN. These discussions ensured that the study was designed appropriately to minimise participant stress and burden, and ensure their safety. The study protocol, participant information sheet and recruitment material have all been reviewed by EDANZ and our study advisors. EDANZ has also offered to support in recruitment for the study by posting on their social media platforms and recommending local clinics and services for us to contact.

ETHICS AND DISSEMINATION

Ethics approval

Ethics approval for the study was granted by the Central Health and Disability Ethics Committee (reference number: 21/CEN/212). The study protocol adheres to the ethical guidelines outlined in the Declaration of

Helsinki.[69] All participants will provide written informed consent before participating in the study.

Data management

Each participant in the study will be given a unique deidentified study ID that will be used to label all their data and samples collected throughout the study. All clinical data will be entered and stored in the web-based platform REDCap,[64] which is hosted in secure servers at the University of Auckland. Access to these data will be restricted to the members of the research team. Clinical data will be stored for a minimum period of 10 years. Biological samples (ie, stool and blood samples) will be securely stored for up to 5 years in −80°C freezers at Te Ira Kāwai—Auckland Regional Biobank, with access restricted to members of the research team for the purposes of this study only. All study personnel involved in data and tissue collection will be trained in good clinical practice, study protocol and collection requirements. Participants will have the right to access and correct their personal data without being withdrawn from the study. If a participant withdraws from the study, any samples or data collected prior to withdrawal will continue to be used and included in the study.

Data availability

At the completion of the study, the deidentified postfiltered metagenomic sequencing data will be made publicly available on National Center for Biotechnology Information's Sequence Read Archive. Note that this data set does not contain human DNA sequences. The deidentified clinical data may be made available for future research on valid requests to the Liggins Institute's Data Oversight Committee. Requestors will need to provide a methodologically sound proposal, obtain appropriate ethics approval and sign a Data Access Agreement. The Data Access Agreement will include a commitment to using the data only for the specified proposal, not to attempt to identify any individual participants, to securely store and use the data and to destroy or return the data after completion of the project. Information on data sharing will be provided in the participant information sheet and will be listed in the consent form.

Dissemination

Findings from this study will be communicated to the scientific community through publications in peer-reviewed journals and presentations at relevant conferences and meetings. Study participants will be informed of the study findings as soon as the results become available. Study findings will also be presented to EDANZ and interested participant care providers. In addition, we will communicate our findings with the general public through liaison with the Liggins Institute's communications manager.

Author affiliations

[1]Liggins Institute, The University of Auckland, Auckland, New Zealand
[2]Department of Paediatrics: Child and Youth Health, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
[3]Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland
[4]Department of Psychological Medicine, The University of Auckland, Auckland, New Zealand
[5]Te Ara Hāro, Centre for Infant, Child and Adolescent Mental Health, The University of Auckland, Auckland, New Zealand

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ORCID iDs

Brooke C Wilson http://orcid.org/0000-0002-2213-542X
José G B Derraik http://orcid.org/0000-0003-1226-1956
Benjamin B Albert http://orcid.org/0000-0003-0498-3473
Karen S W Leong http://orcid.org/0000-0002-5733-8665
Ry Y Tweedie-Cullen http://orcid.org/0000-0003-0128-7181
Taygen Edwards http://orcid.org/0000-0001-7619-0398
Tommi Vatanen http://orcid.org/0000-0003-0949-1291
Hiran Thabrew http://orcid.org/0000-0002-8755-6217
Wayne S Cutfield http://orcid.org/0000-0001-6107-3682
Justin M O'Sullivan http://orcid.org/0000-0003-2927-450X

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