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Protocol for Gut Bugs in Anorexia Nervosa: An open-label pilot trial of faecal microbiome transfer to restore the gut microbiome in anorexia nervosa

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Manuscripts

Protocol for Gut Bugs in Anorexia Nervosa

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

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27 ABSTRACT

28
29 **Introduction:** Individuals with anorexia nervosa (AN) harbour distinct gut microbiomes compared to healthy
30 individuals, which are sufficient to induce weight loss and anxiety-like behaviours when transplanted into
31 germ-free mice. We hypothesise that faecal microbiome transfer (FMT) from healthy donors would help
32 restore the gut microbiome of individuals with AN, which in turn, may aid patient recovery.

33
34 **Methods:** We aim to conduct an open-label pilot study in 20 females aged 16-25 years who meet the DSM-5
35 criteria for AN and have a BMI 13-19 kg/m². We will recruit four healthy, lean, female donors, aged 18-32
36 years, who will undergo extensive clinical screening prior to stool donation. Faecal microbiota will be
37 harvested from donors and double encapsulated in delayed release, acid-resistant capsules. All participants
38 will receive a single course of 20 FMT capsules (five from each donor) which they can choose to take over
39 two or four consecutive days. Stool and blood samples will be collected from participants over a period of
40 three months to assess their gut microbiome profile, metabolome, intestinal inflammation, and nutritional
41 status. Our primary outcome is a shift in the gut microbiome composition at 3 weeks post-FMT (Bray-Curtis
42 dissimilarity). We will also monitor participants' body composition (DXA scans), eating disorder
43 psychopathology, mental health, and assess their views on, and tolerability of, treatment. All adverse events
44 will be recorded and reviewed by an independent data monitoring committee.

45
46 **Ethics and dissemination:** Ethics approval was provided by the Central Health and Disability Ethics
47 Committee (Ministry of Health, New Zealand, 21/CEN/212). Results will be published in peer-reviewed
48 journals and presented to both scientific and consumer group audiences.

49
50 **Trial registration:** Australian New Zealand Clinical Trials Registry (ACTRN12621001504808).

51 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 52 • This pilot trial investigates FMT as a therapy for gut microbiome restoration in young women with AN.
- 53 • This study has been co-designed in consultation with eating disorder specialists and recovered
54 individuals to minimise participant harm and stress.
- 55 • The use of high-resolution shotgun metagenomic sequencing will allow for a comprehensive gut
56 microbiome assessment and longitudinal tracking of donor strain engraftment.
- 57 • While we will monitor clinical features of AN throughout the trial, efficacy of FMT on clinical outcomes
58 cannot be assessed due to its design without a control group.
- 59 • By focusing on young women with AN, study findings may not necessarily apply to the broader population
60 of individuals with AN.

54 INTRODUCTION

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

64
65 Multiple studies have shown that the gut microbiome is perturbed in individuals with AN compared to healthy
66 individuals [14–20]. In particular, anorexia-associated microbiomes are typically less diverse [16, 17, 19]
67 and contain proportionally more mucin-degrading bacterial species such as *Methanobrevibacter smithii* [14,
68 17, 18, 20]. Degradation of the intestinal mucus lining can lead to increased permeability and translocation
69 of bacterial products into circulation [21, 22], both of which have been observed in AN [23–26].

70
71 While the gut microbiome alterations observed in AN are likely a consequence of severe caloric restriction
72 and psychological stress, the gut microbiome itself has also been suggested to play a role in perpetuating
73 symptoms of the disorder [27]. For example, individuals with AN have higher blood levels of caseinolytic-
74 protease-B (ClpB), a protein produced by commensal gut species [26]. ClpB shares homology with the
75 human anorexigenic α -melanocyte-stimulating hormone (α -MSH) and may therefore mimic its function to
76 suppress appetite and increase energy expenditure [28].

77
78 A contributory role of the gut microbiome in AN symptomatology was demonstrated when germ-free mice
79 were inoculated with the gut microbiome derived from either healthy human donors or donors with AN [29].
80 The mice who received the 'AN microbiome' showed reduced body weight and a concomitant reduction in
81 food intake. Interestingly, when the 'AN microbiome' mice ate the same amount of food as the 'healthy
82 microbiome' mice, they gained less weight suggesting they struggled to convert food into body mass [29].
83 Furthermore, the 'AN microbiome' mice had reduced serotonin levels and displayed anxiety-related and
84 compulsive behaviours [29]. Collectively, these findings highlight the gut microbiome as a potential mediator
85 of disease in AN and a suitable target for therapeutic intervention.

86
87 Current treatment approaches for AN are multidisciplinary and focus on nutritional rehabilitation, weight
88 restoration, and cognitive behavioural therapy [30]. However, despite these efforts, approximately 30% of
89 individuals will only partially recover from the disorder, with a further 20% maintaining a chronic course of
90 illness over their lifetime [31]. Even after nutritional interventions and weight restoration, the gut microbiome
91 in individuals with AN remains distinct from that of healthy individuals [16, 17], potentially contributing to
92 relapse of symptoms [27]. Therefore, strategies designed to restore the gut microbiome in AN may be of
93 clinical benefit when used in conjunction with current nutritional rehabilitation therapies, and warrant further
94 investigation.

1
2
3 95
4 96 Faecal microbiome transfer (FMT) involves the transfer of gut microbiota from healthy donors to recipients
5 97 with gut dysbiosis. This therapy has proven highly effective for treating recurrent *Clostridioides difficile*
6 98 infections, and can rapidly restore the diversity and functions of the gut microbiome in these patients [32].
7 99 FMT has also been trialled in other disorders associated with less severe forms of gut dysbiosis, such as
8 100 obesity [33–35], metabolic syndrome [36–39], inflammatory bowel disease [40], irritable bowel syndrome
9 101 [41], and autism [42]. While FMT cannot cure these multi-faceted conditions, its ability to alter the gut
10 102 microbiome has led to various therapeutic benefits among recipients including improvements in fat
11 103 distribution [35], metabolic syndrome [35], insulin sensitivity [36, 37], intestinal permeability [43], gut
12 104 inflammation [44], gastrointestinal symptoms [45], and social behaviours [42].
13 105

14 106 Given the role of the gut microbiome in regulating appetite, mood, and metabolism [10–12], restoring the
15 107 gut microbiome in individuals with AN may act as a stepping stone towards improved patient recovery. There
16 108 have been two published case reports of FMT in patients with AN [46, 47]. In both instances, the patients
17 109 showed an increase in gut microbiome diversity following FMT, however, metabolic improvements and
18 110 weight restoration were only observed in one case [46]. Further research is therefore necessary to better
19 111 understand whether FMT represents a viable treatment option for individuals with AN.
20 112

21 113 The aim of this pilot study is to assess the feasibility of using FMT to help restore the gut microbiome in
22 114 individuals with AN. Rather than using invasive FMT administration approaches with limited scalability, our
23 115 study will employ validated methods for donor microbiome encapsulation [48, 49]. To help boost microbiome
24 116 diversity, participants will receive an equal number of capsules from four donors who will be selected after
25 117 extensive health and microbiome screening. Participants will be monitored for adverse events, have their
26 118 gut microbiome profiled, and be clinically assessed for up to three months post-FMT.
27 119

28 120 **METHODS**

29 121 **Study design**

30 122 This study is a one-arm, open-label pilot trial investigating the safety, tolerability, and potential of FMT to
31 123 restore the gut microbiome in young females with AN. The study will be conducted at the Liggins Institute's
32 124 Clinical Research Unit (University of Auckland), in Auckland, New Zealand.
33 125

34 126 **Participants**

35 127 We aim to recruit 20 female participants aged 16–25 years who meet the DSM-5 criteria for AN and have a
36 128 body mass index (BMI) 13–19 kg/m² (Table 1). Participants will be recruited through engagement with local
37 129 eating disorder clinics, the Eating Disorders Association New Zealand (EDANZ), and social media. Study
38 130 brochures and a detailed participant information sheet will be supplied to potential participants and their
39 131 caregivers who are interested and considered eligible by their specialist physician. Participants will have the
40 132 opportunity to ask any questions about the study before they decide to consent. Participants will be able to
41 133 withdraw from the study at any time.
42 60

Table 1. Eligibility criteria for participants and donors.

	Participants	Donors
Inclusion		
	Biological female at birth	Biological female at birth
	16 - 25 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 ^a	Total body fat ≤33% (as assessed by DXA)
	Medically stable ^b	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 two days)
Exclusion		
	Known allergies to food and/or common medications	Positive screening test for any transmissible pathogen or multi-drug resistant organism listed in Table 2
	Use of antibiotics or probiotics in the month prior to treatment	Gastrointestinal disease (e.g. inflammatory bowel disease, irritable bowel syndrome, coeliac disease, eosinophilic oesophagitis)
	Regular oral steroid treatment or daily application of potent topical steroids extensively to the body	Metabolic disorder (e.g. diabetes, metabolic syndrome, hypertension, dyslipidemia, dysglycaemia)
	Compromised immune system	Impaired fasting glucose (>5.9 mmol/l) or elevated HbA1c (>41 mmol/mol)
	Any chronic illness affecting gut or metabolic health	Asthma or eczema requiring regular prophylaxis or treatment
	Thoughts of self-harm and/or suicide ideation ^c	Autism spectrum conditions
	Current or planned pregnancy during the course of the study	Previous diagnosis of mental health issues including eating disorders
		Current or past history of malignancy
		Use of oral antibiotics or probiotic supplements in the past 3 months
		Regular binge drinking (>5 alcoholic standards/session at least once/week)
		Past or present use of recreational drugs, tobacco, or vaping
		Current or past pregnancy
		Overseas travel in the past two weeks ^d
		UK residence in 1980-1996 for 6 months or longer ^e

^a Meeting the DSM-5 criteria, 307.1

^b In accordance with Starship Children's Hospital's clinical guidelines [50] (i.e., resting heart rate > 50 bpm; no postural drop in blood pressure or rise in heart rate, no electrolyte abnormalities, temperature >35°C and <37.8°C).

^c Based on response to question 9 of the Patient Health Questionnaire-9 (PHQ9) [51].

^d Donors who have travelled overseas will need to wait a minimum period of 2 weeks from their arrival back in New Zealand before donating.

^e Due to the risk of variant Creutzfeldt-Jakob disease.

134 Donors

135 To minimise treatment heterogeneity, we will attempt to use the same four FMT donors throughout the
 136 trial. We aim to recruit up to eight female stool donors to ensure we have sufficient reserve donors if one
 137 becomes unwell, unavailable, or otherwise ineligible during the study. Donors will be recruited through
 138 the University of Auckland's internal email list and via social media advertising. Potential donors will be
 139 interviewed over the phone to assess general eligibility criteria (i.e., health and lifestyle parameters)
 140 before being invited to our clinic for further screening. We will employ the same donor screening protocol
 141 used in our previous FMT trial [52] (Table 1). Donors will be screened to ensure the absence of disease
 142 and any transmissible viral, bacterial, or protozoal pathogens (Table 2). Donors will be given a detailed
 143 participant information sheet to read, and will have the opportunity to ask any questions about the study
 144 before they decide to consent. Donors will be able to withdraw from the study at any time. Any capsules
 145 produced prior to withdrawal may be kept for use in the study.

146
147 **Table 2.** Pathogen screening for donors.

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

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

150 Treatment

151 All participants will receive the same treatment. We will use a multi-donor FMT approach in which
 152 recipients receive 20 capsules containing the gut microbiota from four healthy female donors (5 capsules
 153 from each donor). Treatment will be spread over two days (10 capsules/day) or four days (5 capsules/day)
 154 depending on the participant's preference. The total FMT dose corresponds to 10 g of concentrated gut
 155 microbiota (2.5 g/donor).

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4 157 *FMT capsule preparation*
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6 158 Donors will be asked to visit the Clinical Research Unit every six months to provide fresh stool samples
7 159 for capsule production. Pathogen screening will be repeated for every capsule batch (Table 2). If multiple
8 160 stool samples from the same donor are required for one batch of capsules, repeat screening will only be
9 161 performed if it has been >2 weeks since their last pathogen screen. If any of the four selected donors fail
10 162 their repeat screening, we will contact and invite one of the reserve donors for rescreening.
11
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13 163

14 164 We will use validated methods for gut microbiome encapsulation as described previously [52]. Donor
15 165 stools will be processed individually for encapsulation. Immediately after donation, stool will be blended
16 166 with 1:2 volumes of 0.9% saline solution and sieved to remove particulate matter. To remove any
17 167 remaining particulate matter, the faecal slurry will be centrifuged (200x gravity, 5 min, room temperature).
18 168 The resulting supernatant will be decanted into a fresh tube and centrifuged (5000x gravity, 15 min, room
19 169 temperature) to concentrate the bacterial pellet. The bacterial pellet will be resuspended at a
20 170 concentration of 1 g/ml in a cryoprotective solution (15% glycerol, 0.9% saline) and 500 µl aliquots will
21 171 be dispensed into size 0 delayed release capsules (DRcaps™, Capsugel, Sydney, Australia). The size 0
22 172 capsules will be closed and secondarily sealed in size 00 DRcaps™ capsules. DRcaps™ are specifically
23 173 designed to mask taste and odour, resist stomach acid, and deliver their contents to the proximal bowel
24 174 [53]. Capsules will be stored at -80°C for up to 6 months.
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31 176 *FMT capsule administration*

32 177 Treatment appointments will be scheduled for early morning and the participant will need to have fasted
33 178 overnight for at least 8 hours. Given the high rates of laxative abuse in individuals with AN [54], we will
34 179 not be performing a laxative bowel cleanse prior to FMT. Depending on the participant's preference, the
35 180 capsule dose can be spread over two or four consecutive mornings. Capsules will be swallowed with
36 181 water under direct supervision from a research nurse or clinician. Participants will be asked to postpone
37 182 their breakfast until one hour after swallowing the capsules to help minimise the length of time the
38 183 capsules will spend in the stomach.
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45 185 **Study schedule**

46 186 Participants will have an enrolment appointment where we will explain the study in detail, assess their
47 187 eligibility, and obtain their written informed consent. During this visit, we will perform a whole-body dual-
48 188 energy X-ray absorptiometry (DXA) scan and administer the Patient Health Questionnaire 9 (PHQ-9) to
49 189 confirm they meet the BMI criteria and do not have feelings of self-harm/suicidal ideation. We will also
50 190 collect a stool sample from the participant during this visit and schedule their baseline assessment for 3
51 191 weeks time (Table 3). The first treatment dose will be scheduled on the same day or within a few days of
52 192 their baseline assessment. Follow-up clinical assessments will be scheduled for 6 and 12 weeks after
53 193 treatment. Participants will also be asked to collect a stool sample at home 3 weeks after their baseline
54 194 assessment.
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196 **Table 3.** Schedule of enrolment, treatment, and clinical assessments for study participants.

	Enrolment 3 weeks before baseline	Baseline clinical assessment	Treatment spread over 2 or 4 days	1- week	3- week	6-week clinical assessment	12-week clinical assessment
Eligibility screen	✓						
Informed consent	✓						
FMT treatment			✓				
Adverse events			✓	✓	✓	✓	✓
Tolerability questionnaire			✓				
Background questionnaire		✓					
Eating disorder symptoms (EDEQ)		✓				✓	✓
Depression symptoms (PHQ-9)	✓	✓				✓	✓
Anxiety symptoms (GAD-7)		✓				✓	✓
Stool sample	✓	✓			✓	✓	✓
Blood sample		✓				✓	✓
Body composition scan (DXA)	✓						✓

197 DXA, dual-energy X-ray absorptiometry; EDEQ, Eating Disorder Examination Questionnaire; FMT, faecal microbiome transfer;
 198 GAD-7, General Anxiety Disorder 7-item scale; PHQ-9, Patient Health Questionnaire 9.

200 Data collection and follow-up

201 *Stool sample collection*

202 Stool samples will be collected from participants 3 weeks before treatment, at baseline, and 3, 6, and 12
 203 weeks after treatment to assess changes in the gut microbiome, metabolome, and levels of intestinal
 204 inflammation. Where possible, stool samples will be collected on site except for the “3 weeks before” and
 205 “3 weeks after” treatment samples which the participant will collect at home. If a participant cannot
 206 produce a stool sample during their clinical assessment visit, they will be given a stool collection kit to
 207 take home. Collection kits contain a stool catcher, disposable gloves, specimen bag, specimen pottle,
 208 DNA/RNA Shield Fecal Collection tube (#R1101, Zymo Research, Irvine, California, USA), and a step-
 209 by-step instruction card. If the sample is collected at home, participants will be asked to store their
 210 samples within their home freezer until their next appointment or arrange for collection by a member of
 211 the research team. Upon receipt, stool samples will be aliquoted and stored in -80°C freezers at Te Ira
 212 Kāwai – Auckland Regional Biobank. Stool collected in the DNA/RNA Shield Fecal Collection tube will be
 213 reserved for gut microbiome assessment. Stool collected in the specimen pottle (i.e. not containing any
 214 stabilization buffer) will be reserved for metabolomics and intestinal inflammation assays (e.g.
 215 calprotectin, lactoferrin, and S100A12 [55]).

217 *Gut microbiome profiling*

218 DNA and RNA will be extracted using the ZymoBIOMICS MagBead DNA/RNA kit (Zymo Research,
 219 #R2136) according to the manufacturer’s instructions with the addition of a bead bashing lysis step (Zymo
 220 Research, #S6002-96-3). Shotgun metagenomic and metatranscriptomic sequencing will be performed
 221 by a commercial provider using Illumina’s paired-end sequencing technology. Sequencing data will be
 222 processed as performed previously [49], using bioBakery tools for meta’omic profiling [56]. In particular,

223 StrainPhiAn [57] will be used to generate single nucleotide polymorphism (SNP) haplotypes representing
 224 the dominant strain of any given species within a sample. We will use these SNP haplotypes to compare
 225 the genetic similarity of donor and recipient strains before and after treatment to assess the proportion
 226 and stability of donor strain engraftment.

228 *Blood sample collection*

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

234 **Table 4.** Blood test schedule for all clinical assessment visits (baseline, week 6, week 12).

	Safety monitoring	Study outcomes
Electrolytes (Sodium, Potassium)	✓	✓
Creatinine	✓	✓
Ferritin	✓	✓
Total protein	✓	✓
Albumin	✓	✓
Alkaline phosphatase (ALP)	✓	✓
Alanine aminotransferase (ALT)	✓	✓
Gamma-glutamyl transferase (GGT)	✓	✓
Aspartate aminotransferase (AST)		✓
Folate		✓
Vitamin B12		✓
Cortisol		✓
Free thyroxine		✓
Thyroid stimulating hormone (TSH)		✓
Serotonin (5-HT)		✓

236 *Anthropometry and body composition*

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

247 *Questionnaires*

248 All questionnaires will be completed by the participants online using data capture tools from the web-
 249 based research platform, REDCap (Research Electronic Data Capture), hosted in secure servers at the

1
2
3 250 University of Auckland. At the beginning of the study, we will collect background demographic information
4 251 from participants including their age, sex assigned at birth, gender identity, self-reported ethnicity,
5 252 socioeconomic status (based on physical address), age when first diagnosed with AN, and current
6 253 medications. Socioeconomic status will be estimated using the New Zealand Indices of Multiple
7 254 Deprivation [58].
8
9

10 255
11 256 After their final dose of capsules, participants will complete a short questionnaire to gather their views
12 257 and experience of taking the treatment. Specifically, participants will be asked how difficult it was to
13 258 swallow the capsules, whether they experienced any unpleasant side effects during and/or after
14 259 swallowing the capsules, and whether they would consider taking the treatment again if it was later shown
15 260 to be beneficial for recovery.
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19 261
20 262 Participants will also complete three established health questionnaires at baseline, 6 weeks, and 12
21 263 weeks after treatment; 1) Eating Disorder Examination Questionnaire (EDEQ v6.0) [59], 2) Patient Health
22 264 Questionnaire 9 (PHQ-9) for symptoms of depression [51], and 3) General Anxiety Disorder 7-item scale
23 265 (GAD-7) [60].
24
25
26

27 266
27 267 **Safety monitoring**
28
29 268 By adopting strict selection criteria for donors, we will reduce the risk of infection via FMT by minimising
30 269 the potential transmission of pathogenic organisms. Participants will take each dose of FMT in our clinic
31 270 under the supervision of a research clinician and/or nurse, where they will remain under close monitoring
32 271 for at least one hour afterwards. Based on our previous experience and existing evidence, it is unlikely
33 272 that participants will experience any severe adverse events [35]. However, participants will be instructed
34 273 to seek immediate medical attention if they develop any severe adverse reactions following treatment.
35 274 We will contact participants 24 hours after ingestion of each set of capsules, as well as 1, 3, 6, and 12
36 275 weeks after treatment to enquire about any adverse side effects. Specifically, participants will be asked
37 276 to report on the following events: loose or bloody stools, abdominal pain, vomiting, nausea, constipation,
38 277 flatulence, bloating, fever, malodorous burps, flu-like symptoms, allergic symptoms, appetite, fatigue, and
39 278 agitation. Adverse events will be graded in accordance with Common Terminology Criteria for Adverse
40 279 Events v4.0 (CTCAE)[61].
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47 280
47 281 In addition, we will monitor blood markers of nutritional status and liver function (Table 4), any available
48 282 body weight records as provided by the participant's clinical care team, and questionnaire scores
49 283 throughout the study in case any of the participants' health starts deteriorating. If the participant answers
50 284 "several days", "more than half the days" or "nearly every day" to PHQ-9, question 9 "Thoughts that you
51 285 would be better off dead or of hurting yourself in some way", the research clinician will interview the
52 286 participant further and provide them with safety management information to take home. Before the
53 287 participant leaves the clinic, the research clinician will also recommend clinical follow-up and contact the
54 288 participant's routine care provider and/or the research psychiatrist to ensure additional mental health
55 289 support is provided.
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290 These clinical and adverse event data will be reviewed by an independent data monitoring committee
291 (DMC) who can decide to stop the trial if the safety of participants is thought to have been compromised.
292 Any serious adverse event or clinical result will be notified immediately to the DMC.

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

300 **Outcomes**

301 *Primary outcome*

- 302 • A shift in gut microbiome composition at 3 weeks post-FMT. The shift should exceed the drift in
303 gut microbiome composition measured over the 3 weeks between enrolment and baseline.

305 *Secondary outcomes*

- 306 • Adverse events associated with FMT treatment
- 307 • Proportion of participants who swallow all 20 treatment capsules
- 308 • Proportion of participants who would consider having the treatment again if effective
- 309 • Gut microbiome composition and functional potential at 6, and 12 weeks post-FMT
- 310 • Donor strain engraftment at 3, 6, and 12 weeks post-FMT
- 311 • Intestinal inflammation at 6 and 12 weeks post-FMT
- 312 • Blood markers of nutritional status and liver/thyroid function at 6 and 12 weeks post-FMT
- 313 • Eating disorder symptoms at 6 and 12 weeks post-FMT
- 314 • Depression symptoms at 6 and 12 weeks post-FMT
- 315 • Anxiety symptoms at 6 and 12 weeks post-FMT
- 316 • BMI at 12 weeks post-FMT
- 317 • Body composition at 12 weeks post-FMT

319 **Sample size calculation**

320 Our primary outcome is a shift in the gut microbiome composition at 3 weeks post-FMT. Because we do
321 not have a control group to compare against, we will instead collect a stool sample 3 weeks prior to FMT
322 to assess the background drift in the gut microbiome over a 3 week period without any intervention. We
323 will use the Bray-Curtis dissimilarity index to compare gut microbiome composition profiles between
324 sampling time points and test for a difference in these values using a paired t-test. To identify a shift in
325 the gut microbiome above background drift, we will need 18 participants (80% power, alpha = 0.05). This
326 calculation was based on data from our previous FMT trial [35] comparing the gut microbiome shifts
327 between 39 FMT and 44 placebo recipients over 6 weeks (Bray-Curtis dissimilarity to baseline; FMT
328 mean 0.574, Placebo mean 0.416, delta = 0.158, SD = 0.163, t-test p = 2.754e-06). To account for a
329 potential dropout rate of 10%, we aim to recruit 20 participants.

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4 331 **Statistical analyses**
5
6 332 We will perform both intention-to-treat and per-protocol analyses. Per-protocol analyses will only include
7 333 data from those that complete the full treatment dose. Baseline demographics and clinical characteristics
8 334 will be summarized using descriptive statistics. Gut microbiome shifts will be assessed by calculating the
9 335 Bray-Curtis dissimilarity index to and from baseline using species-level relative abundance profiles. To
10 336 assess the primary outcome, a two-sided paired t-test will compare the potential shift in gut microbiome
11 337 composition 3 weeks before treatment to the shift 3 weeks after treatment. No imputation will be
12 338 performed for missing data, and statistical significance will be set at $p < 0.05$.

13 339
14 340 Multivariate Association with Linear Models (MaAsLin2) will be used to examine changes in the relative
15 341 abundances of microbial taxa and their encoded functions in response to treatment. We will also use
16 342 MaAsLin2 to explore associations between microbiome features and clinical outcomes.

17 343
18 344 Changes in clinical outcomes from baseline will be assessed using paired t-tests (parametric) or Wilcoxon
19 345 signed rank tests (non-parametric), as appropriate. However, we acknowledge that we cannot make any
20 346 treatment efficacy claims based on these paired within-group analyses and without a control group.

21 347
22 348 **Patient and public involvement**
23 349 This study has been co-designed in consultation with members from the Eating Disorders Association
24 350 New Zealand (EDANZ) as well as women who have previously recovered from AN. These discussions
25 351 ensured the study was designed appropriately to minimise participant stress and burden. The study
26 352 protocol, participant information sheet, and recruitment material have all been reviewed by EDANZ and
27 353 our study advisors. EDANZ has also offered to support in recruitment for the study by posting on their
28 354 social media platforms and recommending local clinics and services for us to contact.

29 355

30 356 **ETHICS AND DISSEMINATION**

31 357 **Ethics approval**

32 358 Ethics approval for the study was granted by the Central Health and Disability Ethics Committee
33 359 (reference number: 21/CEN/212). The study protocol adheres to the ethical guidelines outlined in the
34 360 Declaration of Helsinki [62]. All participants will provide written informed consent before participating in
35 361 the study.

36 362
37 363 **Data management**
38 364 Each participant in the study will be given a unique de-identified study ID that will be used to label all their
39 365 data and samples collected throughout the study. All recorded clinical data will be entered and stored in
40 366 the web-based platform REDCap, which is hosted in secure servers at the University of Auckland. Access
41 367 to these data will be restricted to the members of the research team. Clinical data will be stored for a
42 368 minimum period of 10 years. Biological samples (i.e., stool and blood samples) will be securely stored for
43 369 up to five years in -80°C freezers at Te Ira Kāwai - Auckland Regional Biobank, with access restricted to

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3 370 members of the research team for the purposes of this study only. All study personnel involved in data
4 371 and tissue collection will be trained in good clinical practice (GCP), study protocol, and collection
5 372 requirements. Participants will have the right to access and correct their personal data without being
6 373 withdrawn from the study. If a participant withdraws from the study, any samples or data collected prior
7 374 to withdrawal will continue to be used and included in the study.
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11 376 **Data availability**

12 377 At the completion of the study, the de-identified post-filtered metagenomic sequencing data will be made
13 378 publicly available on NCBI's sequence read archive (SRA). Note that this data set does not contain human
14 379 DNA sequences. The de-identified clinical data may be made available for future research upon valid
15 380 requests to the Liggins Institute Clinical Data Research Hub Data Access Committee. Requestors will
16 381 need to provide a methodologically sound proposal, obtain appropriate ethics approval, and sign a Data
17 382 Access Agreement. The Data Access Agreement will include a commitment to using the data only for the
18 383 specified proposal, not to attempt to identify any individual participants, to securely store and use the
19 384 data, and to destroy or return the data after completion of the project. Information on data sharing will be
20 385 provided in the participant information sheet and will be listed in the consent form.
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27 387 **Dissemination**

28
29 388 Findings from this study will be communicated to the scientific community through publications in peer-
30 389 reviewed journals and presentations at relevant conferences and meetings. Study participants will be
31 390 informed of the study findings as soon as the results become available. Study findings will also be
32 391 presented to EDANZ and interested participant care providers. In addition, we will communicate our
33 392 findings with the general public through liaison with the Liggins Institute's communications manager.
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39
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44 399 the study framework with Māori perspectives. We are very grateful to the Rockfield Trust for their
45 400 generous donation to fund this study.
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51 402 **AUTHOR CONTRIBUTIONS**

52
53 403 Funding acquisition: WC, JO

54 404 Consultation: BW, JD, BA, KL, CC, MD, HT, WC, JO

55 405 Study design: BW, JD, BA, KL, RT, CC, MD, TE, TV, HT, WC, JO

56 406 Ethics application: BW, JD, BA, KL, TE, TV, HT, WC, JO

57 407 Protocol drafting: BW

58 408 Protocol revision: BW, JD, BA, KL, RT, CC, MD, TE, TV, HT, WC, JO
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5 410 **CONFLICTS OF INTERESTS**

6
7 411 The authors have no conflicts of interest to declare.

8 412

9
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11
12 414 This study is fully funded by the Rockfield Trust. The funders had no involvement in the design of the
13 415 study, and will have no involvement in the collection, analyses, interpretation of data, or in the writing or
14 416 decision to publish the manuscript on study findings.
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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 14

1	Roles and	#5b	Name and contact information for the trial sponsor	1
2	responsibilities:			
3	sponsor contact			
4	information			
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8	Roles and	#5c	Role of study sponsor and funders, if any, in study	14
9	responsibilities:		design; collection, management, analysis, and	
10	sponsor and funder		interpretation of data; writing of the report; and the	
11			decision to submit the report for publication, including	
12			whether they will have ultimate authority over any of	
13			these activities	
14				
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17	Roles and	#5d	Composition, roles, and responsibilities of the	10
18	responsibilities:		coordinating centre, steering committee, endpoint	
19	committees		adjudication committee, data management team, and	
20			other individuals or groups overseeing the trial, if	
21			applicable (see Item 21a for data monitoring committee)	
22				
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26	Introduction			
27				
28	Background and	#6a	Description of research question and justification for	3,4
29	rationale		undertaking the trial, including summary of relevant	
30			studies (published and unpublished) examining benefits	
31			and harms for each intervention	
32				
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34				
35	Background and	#6b	Explanation for choice of comparators	11
36	rationale: choice of			
37	comparators			
38				
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40	Objectives	#7	Specific objectives or hypotheses	11
41				
42				
43	Trial design	#8	Description of trial design including type of trial (eg,	4
44			parallel group, crossover, factorial, single group),	
45			allocation ratio, and framework (eg, superiority,	
46			equivalence, non-inferiority, exploratory)	
47				
48				
49	Methods:			
50	Participants,			
51	interventions, and			
52	outcomes			
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55				
56	Study setting	#9	Description of study settings (eg, community clinic,	4
57			academic hospital) and list of countries where data will	
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be collected. Reference to where list of study sites can be obtained

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4	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
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11	Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
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15			
16	Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)
17			7, 10
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23	Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)
24			7, 10
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28	Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
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32	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
33			11
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43	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
44			8
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50	Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
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57	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size
58			4,6
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1 **Methods:**

2 **Assignment of**
3 **interventions (for**
4 **controlled trials)**
5
6

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8	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,
9	generation		computer-generated random numbers), and list of any
10			factors for stratification. To reduce predictability of a
11			random sequence, details of any planned restriction (eg,
12			blocking) should be provided in a separate document
13			that is unavailable to those who enrol participants or
14			assign interventions
15			
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19	Allocation	#16b	Mechanism of implementing the allocation sequence (eg,
20	concealment		central telephone; sequentially numbered, opaque,
21	mechanism		sealed envelopes), describing any steps to conceal the
22			sequence until interventions are assigned
23			
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26	Allocation:	#16c	Who will generate the allocation sequence, who will
27	implementation		enrol participants, and who will assign participants to
28			interventions
29			
30			
31	Blinding (masking)	#17a	Who will be blinded after assignment to interventions
32			(eg, trial participants, care providers, outcome
33			assessors, data analysts), and how
34			
35			
36	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is
37	emergency unblinding		permissible, and procedure for revealing a participant's
38			allocated intervention during the trial
39			
40			

41 **Methods: Data**
42 **collection,**
43 **management, and**
44 **analysis**
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48	Data collection plan	#18a	Plans for assessment and collection of outcome,
49			baseline, and other trial data, including any related
50			processes to promote data quality (eg, duplicate
51			measurements, training of assessors) and a description
52			of study instruments (eg, questionnaires, laboratory
53			tests) along with their reliability and validity, if known.
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Reference to where data collection forms can be found, if not in the protocol

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4	Data collection plan:	#18b	Plans to promote participant retention and complete
5	retention		follow-up, including list of any outcome data to be
6			collected for participants who discontinue or deviate from
7			intervention protocols
8			
9			
10	Data management	#19	Plans for data entry, coding, security, and storage,
11			including any related processes to promote data quality
12			(eg, double data entry; range checks for data values).
13			Reference to where details of data management
14			procedures can be found, if not in the protocol
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19	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary
20			outcomes. Reference to where other details of the
21			statistical analysis plan can be found, if not in the
22			protocol
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26	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and
27	analyses		adjusted analyses)
28			
29			
30	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-
31	population and		adherence (eg, as randomised analysis), and any
32	missing data		statistical methods to handle missing data (eg, multiple
33			imputation)
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36	Methods: Monitoring		
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38			
39	Data monitoring:	#21a	Composition of data monitoring committee (DMC);
40	formal committee		summary of its role and reporting structure; statement of
41			whether it is independent from the sponsor and
42			competing interests; and reference to where further
43			details about its charter can be found, if not in the
44			protocol. Alternatively, an explanation of why a DMC is
45			not needed
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50	Data monitoring:	#21b	Description of any interim analyses and stopping
51	interim analysis		guidelines, including who will have access to these
52			interim results and make the final decision to terminate
53			the trial
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57	Harms	#22	Plans for collecting, assessing, reporting, and managing
58			solicited and spontaneously reported adverse events
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and other unintended effects of trial interventions or trial conduct

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4	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
5			n/a
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9	Ethics and		
10	dissemination		
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13	Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval
14			13
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17	Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)
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24			
25	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
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30	Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
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36	Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
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43	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site
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47	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
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52	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
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57	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the
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public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions

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5	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of
6	authorship		professional writers
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9	Dissemination policy:	#31c	Plans, if any, for granting public access to the full
10	reproducible research		protocol, participant-level dataset, and statistical code
11			
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13 Appendices

14			
15	Informed consent	#32	Model consent form and other related documentation
16	materials		given to participants and authorised surrogates
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19	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of
20			biological specimens for genetic or molecular analysis in
21			the current trial and for future use in ancillary studies, if
22			applicable
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BMJ Open

Protocol for Gut Bugs in Anorexia Nervosa: An open-label pilot trial of faecal microbiome transfer to restore the gut microbiome in anorexia nervosa

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Manuscripts

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5 2 **An open-label pilot trial of faecal microbiome transfer to restore the gut**
6 3 **microbiome in anorexia nervosa**
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27 ABSTRACT

28

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

33

34 **Methods:** We aim to conduct an open-label pilot study in 20 females aged 16-32 years who meet the DSM-5
35 criteria for AN and have a BMI 13-19 kg/m². We will recruit four healthy, lean, female donors, aged 18-32
36 years, who will undergo extensive clinical screening prior to stool donation. Faecal microbiota will be
37 harvested from donors and double encapsulated in delayed release, acid-resistant capsules. All participants
38 will receive a single course of 20 FMT capsules (five from each donor) which they can choose to take over
39 two or four consecutive days. Stool and blood samples will be collected from participants over a period of
40 three months to assess their gut microbiome profile, metabolome, intestinal inflammation, and nutritional
41 status. Our primary outcome is a shift in the gut microbiome composition at 3 weeks post-FMT (Bray-Curtis
42 dissimilarity). We will also monitor participants' body composition (DXA scans), eating disorder
43 psychopathology, mental health, and assess their views on, and tolerability of, treatment. All adverse events
44 will be recorded and reviewed by an independent data monitoring committee.

45

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

49

50 **Trial registration:** Australian New Zealand Clinical Trials Registry (ACTRN12621001504808).

51

52

53 STRENGTHS AND LIMITATIONS OF THIS STUDY

- This pilot trial investigates FMT as a therapy for gut microbiome restoration in young women with AN.
- This study has been co-designed 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.

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54 INTRODUCTION

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

64
65 Multiple studies have shown that the gut microbiome is perturbed in individuals with AN [14–25]. Early
66 reports suggested AN microbiomes were typically less diverse when compared against healthy age-
67 matched controls [19, 21]. However, more recent observations do not support a simple reduction in microbial
68 diversity being linked to AN [16, 17, 24], but rather, a difference in the relative abundances of specific taxa.
69 In particular, a recent systematic review determined that AN individuals harboured proportionally less fiber-
70 utilising taxa (e.g. *Roseburia* sp.) and more mucin-degrading taxa (e.g. *Akkermansia* sp. and
71 *Methanobrevibacter smithii*) [26]. Degradation of the intestinal mucus lining can lead to increased
72 permeability and translocation of bacterial products into circulation [27, 28], both of which have been
73 observed in AN [29–32].

74
75 While the gut microbiome alterations observed in AN are likely a consequence of severe caloric restriction
76 and psychological stress, the gut microbiome itself has also been suggested to play a role in perpetuating
77 symptoms of the disorder [33]. For example, individuals with AN have higher blood levels of caseinolytic-
78 protease-B (ClpB), a protein produced by commensal gut species [32]. ClpB shares homology with the
79 human anorexigenic α -melanocyte-stimulating hormone (α -MSH) and may therefore mimic its function to
80 suppress appetite and increase energy expenditure [34].

81
82 A contributory role of the gut microbiome in AN symptomatology was demonstrated when germ-free mice
83 were inoculated with the gut microbiome derived from either healthy human donors or donors with AN [35].
84 The offspring of mice who received the 'AN microbiome' showed reduced body weight and a concomitant
85 reduction in food intake. Interestingly, when the 'AN microbiome' mice ate the same amount of food as the
86 'healthy microbiome' mice, they gained less weight suggesting they were less efficient at converting food
87 into body mass [35]. Furthermore, the 'AN microbiome' mice had reduced serotonin levels and displayed
88 anxiety-related and compulsive behaviours [35]. Similar weight gain differences were observed in another
89 germ-free mice experiment that utilised both AN and healthy control donors, with differences being linked
90 to altered expression of appetite-suppression and thermogenesis genes [17]. By contrast, another study
91 found no difference in body weight or daily food intake between mice receiving transplantations of AN- or
92 healthy-donor microbiota [36]. Further research is therefore required to determine whether the gut
93 microbiome acts as a potential mediator of disease in AN and is thus a suitable target for therapeutic
94 intervention.

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4 96 Current treatment approaches for AN are multidisciplinary and focus on nutritional rehabilitation, weight
5 97 restoration, and cognitive behavioural therapy [37]. However, despite these efforts, approximately 30% of
6 98 individuals will only partially recover from the disorder, with a further 20% maintaining a chronic course of
7 99 illness over their lifetime [38]. Even after nutritional interventions and weight restoration, the gut microbiome
8 100 in individuals with AN remains distinct from that of healthy individuals [19, 20], potentially contributing to
9 101 relapse of symptoms [33]. Therefore, strategies designed to restore the gut microbiome in AN may be of
10 102 clinical benefit when used in conjunction with current nutritional rehabilitation therapies, and warrant further
11 103 investigation.
12 104

13 105 Faecal microbiome transfer (FMT) involves the transfer of gut microbiota from healthy donors to recipients
14 106 with gut dysbiosis. This therapy has proven highly effective for treating recurrent *Clostridioides difficile*
15 107 infections, and can rapidly restore the diversity and functions of the gut microbiome in these patients [39].
16 108 FMT has also been trialled in other disorders associated with less severe forms of gut dysbiosis, such as
17 109 obesity [40–42], metabolic syndrome [43–46], inflammatory bowel disease [47], irritable bowel syndrome
18 110 [48], and autism [49]. While FMT cannot cure these multi-faceted conditions, its ability to alter the gut
19 111 microbiome has led to various therapeutic benefits among recipients including improvements in fat
20 112 distribution [42], metabolic syndrome [42], insulin sensitivity [43, 44], intestinal permeability [50], gut
21 113 inflammation [51], gastrointestinal symptoms [52], and social behaviours [49].
22 114

23 115 Given the role of the gut microbiome in regulating appetite, mood, and metabolism [10–12], restoring the
24 116 gut microbiome in individuals with AN may act as a stepping stone towards improved patient recovery. There
25 117 have been two published case reports of FMT in patients with AN [53, 54]. In both instances, the patients
26 118 showed an increase in gut microbiome diversity following FMT, however, metabolic improvements and
27 119 weight restoration were only observed in one case [53]. Further research is therefore necessary to better
28 120 understand whether FMT represents a viable treatment option for individuals with AN.
29 121

30 122 The aim of this pilot study is to assess the feasibility of using FMT to help restore the gut microbiome in
31 123 individuals with AN. Rather than using invasive FMT administration approaches with limited scalability, our
32 124 study will employ validated methods for donor microbiome encapsulation [55, 56]. To help boost microbiome
33 125 diversity, participants will receive an equal number of capsules from four donors who will be selected after
34 126 extensive health and microbiome screening. Participants will be monitored for adverse events, have their
35 127 gut microbiome profiled, and be clinically assessed for up to three months post-FMT.
36 128

37 129 **METHODS**

38 130 **Study design**

39 131 This study is a one-arm, open-label pilot trial investigating the safety, tolerability, and potential of FMT to
40 132 restore the gut microbiome in young females with AN. The study will be conducted at the Liggins Institute's
41 133 Clinical Research Unit (University of Auckland), in Auckland, New Zealand.
42 134

1
2
3 135 **Participants**

4 136 We aim to recruit 20 female participants aged 16-32 years who meet the DSM-5 criteria for AN and have a
5 137 body mass index (BMI) 13-19 kg/m² (Table 1). Participants will be recruited through engagement with local
6 eating disorder clinics, the Eating Disorders Association New Zealand (EDANZ), and social media. Study
7 138 brochures and a detailed participant information sheet will be supplied to potential participants and their
8 139 caregivers who are interested and considered eligible by their specialist physician. Participants will have the
9 140 opportunity to ask any questions about the study before they decide to consent. Participants will be able to
10 141 withdraw from the study at any time.
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For peer review only

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 ^a	Total body fat ≤33% (as assessed by DXA)
	Medically stable ^b	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 two days)
Exclusion		
	Known allergies to food and/or common medications	Positive screening test for any transmissible pathogen or multi-drug resistant organism listed in Table 2
	Use of antibiotics or probiotics in the month prior to treatment	Gastrointestinal disease (e.g. inflammatory bowel disease, irritable bowel syndrome, coeliac disease, eosinophilic oesophagitis)
	Regular oral steroid treatment or daily application of potent topical steroids extensively to the body	Metabolic disorder (e.g. diabetes, metabolic syndrome, hypertension, dyslipidemia, dysglycaemia)
	Compromised immune system	Impaired fasting glucose (>5.9 mmol/l) or elevated HbA1c (>41 mmol/mol)
	Any chronic illness affecting gut or metabolic health	Asthma or eczema requiring regular prophylaxis or treatment
	Thoughts of self-harm and/or suicide ideation ^c	Autism spectrum conditions
	Current or planned pregnancy during the course of the study	Previous diagnosis of mental health issues including eating disorders
	Regular laxative use	Current or past history of malignancy
		Use of oral antibiotics or probiotic supplements in the past 3 months
		Regular binge drinking (>5 alcoholic standards/session at least once/week)
		Past or present use of recreational drugs, tobacco, or vaping
		Current or past pregnancy
		Overseas travel in the past two weeks ^d
		UK residence in 1980-1996 for 6 months or longer ^e

^a Meeting the DSM-5 criteria, 307.1

^b In accordance with Starship Children's Hospital's clinical guidelines [57] (i.e., resting heart rate > 50 bpm; no postural drop in blood pressure or rise in heart rate, no electrolyte abnormalities, temperature >35°C and <37.8°C).

^c Based on response to question 9 of the Patient Health Questionnaire-9 (PHQ9) [58] .

^d Donors who have travelled overseas will need to wait a minimum period of 2 weeks from their arrival back in New Zealand before donating.

^e Due to the risk of variant Creutzfeldt-Jakob disease.

143 Donors

144 To minimise treatment heterogeneity, we will attempt to use the same four FMT donors throughout the
 145 trial. We aim to recruit up to eight female stool donors to ensure we have sufficient reserve donors if one
 146 becomes unwell, unavailable, or otherwise ineligible during the study. Donors will be recruited through
 147 the University of Auckland's internal email list and via social media advertising. Potential donors will be
 148 interviewed over the phone to assess general eligibility criteria (i.e. health and lifestyle parameters) before
 149 being invited to our clinic for further screening. We will employ the same donor screening protocol used
 150 in our previous FMT trial [59] (Table 1). Donors will be screened to ensure the absence of disease and
 151 any transmissible viral, bacterial, or protozoal pathogens (Table 2). Donors will be given a detailed
 152 participant information sheet to read, and will have the opportunity to ask any questions about the study
 153 before they decide to consent. Donors will be able to withdraw from the study at any time. Any capsules
 154 produced prior to withdrawal may be kept for use in the study.

155 **Table 2.** Pathogen screening for donors.

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

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

159 Treatment

160 All participants will receive the same treatment. We will use a multi-donor FMT approach in which
 161 recipients receive 20 capsules containing the gut microbiota from four healthy female donors (5 capsules
 162 from each donor). Treatment will be spread over two days (10 capsules/day) or four days (5 capsules/day)
 163 depending on the participant's preference. The total FMT dose corresponds to 10 g of concentrated gut
 164 microbiota (2.5 g/donor).

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5 166 *FMT capsule preparation*
6 167 Donors will be asked to visit the Clinical Research Unit every six months to provide fresh stool samples
7 168 for capsule production. Pathogen screening will be repeated for every capsule batch (Table 2). If multiple
8
9 169 stool samples from the same donor are required for one batch of capsules, repeat screening will only be
10 170 performed if it has been >2 weeks since their last pathogen screen. If any of the four selected donors fail
11 171 their repeat screening, we will contact and invite one of the reserve donors for rescreening.
12

13 172
14 173 We will use validated methods for gut microbiome encapsulation as described previously [59]. Donor
15 174 stools will be processed individually for encapsulation under aerobic conditions. Immediately after
16 175 donation, stool will be blended with 1:2 volumes of 0.9% saline solution and sieved to remove particulate
17 176 matter. To remove any remaining particulate matter, the faecal slurry will be centrifuged (200x gravity, 5
18 177 min, room temperature). The resulting supernatant will be decanted into a fresh tube and centrifuged
19 178 (5000x gravity, 15 min, room temperature) to concentrate the bacterial pellet. The bacterial pellet will be
20 179 resuspended at a concentration of 1 g/ml in a cryoprotective solution (15% glycerol, 0.9% saline) and 500
21 180 µl aliquots will be dispensed into size 0 delayed release capsules (DRcaps™, Capsugel, Sydney,
22 181 Australia). The size 0 capsules will be closed and secondarily sealed in size 00 DRcaps™ capsules.
23 182 DRcaps™ are specifically designed to mask taste and odour, resist stomach acid, and deliver their
24 183 contents to the proximal bowel [60]. Capsules will be stored at -80°C for up to 6 months.
25
26 184

185 *FMT capsule administration*

186 Treatment appointments will be scheduled for early morning and the participant will need to have fasted
187 overnight for at least 8 hours. Given the high rates of laxative abuse in individuals with AN [61], we will
188 not be performing a laxative bowel cleanse prior to FMT. Depending on the participant's preference, the
189 capsule dose can be spread over two or four consecutive mornings. Capsules will be swallowed with
190 water under direct supervision from a research nurse or clinician. Participants will be asked to postpone
191 their breakfast until one hour after swallowing the capsules to help minimise the length of time the
192 capsules will spend in the stomach.
193

194 **Study schedule**

195 Participants will have an enrolment appointment where we will explain the study in detail, assess their
196 eligibility, and obtain their written informed consent. During this visit, we will perform a whole-body dual-
197 energy X-ray absorptiometry (DXA) scan and administer the Patient Health Questionnaire 9 (PHQ-9) to
198 confirm they meet the BMI criteria and do not have feelings of self-harm/suicidal ideation. We will also
199 collect a stool sample from the participant during this visit and schedule their baseline assessment for 3
200 weeks time (Table 3). The first treatment dose will be given at baseline after all assessments have been
201 completed. Subsequent treatment doses will be scheduled the following consecutive day/s depending on
202 the participant's preference for a 2-day or 4-day treatment schedule. Follow-up clinical assessments will
203 be scheduled for 6 and 12 weeks after baseline. Participants will also be asked to collect a stool sample
204 at home 3 weeks after their baseline assessment.

205 **Table 3.** Study Schedule.

	Screening & Enrolment	Baseline & Treatment day 1	Treatment day 2*	48 hours	1-week	3-weeks	6-weeks	12-weeks
Scheduling	<i>3 weeks before baseline</i>	<i>3 weeks after enrolment</i>	<i>1 day after baseline</i>	<i>2 days after baseline</i>	<i>1 week after baseline</i>	<i>3 weeks after baseline</i>	<i>6 weeks after baseline</i>	<i>12 weeks after baseline</i>
Visit type	<i>Clinic</i>	<i>Clinic</i>	<i>Clinic</i>	<i>Phone-call</i>	<i>Phone-call</i>	<i>Phone-call</i>	<i>Clinic</i>	<i>Clinic</i>
Eligibility screen	✓							
Informed consent	✓							
FMT treatment		✓	✓					
Adverse events			✓	✓	✓	✓	✓	✓
Tolerability questionnaire			✓					
Background questionnaire		✓						
Eating disorder symptoms (EDEQ)		✓					✓	✓
Depression symptoms (PHQ-9)	✓	✓					✓	✓
Anxiety symptoms (GAD-7)		✓					✓	✓
Stool sample	✓	✓				✓	✓	✓
Blood sample		✓					✓	✓
Body composition scan (DXA)	✓							✓

206 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
 207 Questionnaire 9.
 208 *Study timeline is based on a 2-day treatment schedule. If a participant decides to spread their treatment over 4 consecutive days, the tolerability questionnaire will be administered on the final day of
 209 treatment (day 4) and the 48 hour phone call will be 24 hours after the final dose of capsules.

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3 210 **Data collection and follow-up**

4 211 *Stool sample collection*

5
6 212 Stool samples will be collected from participants 3 weeks before treatment, at baseline, and 3, 6, and 12
7 213 weeks after treatment to assess changes in the gut microbiome, metabolome, and levels of intestinal
8 214 inflammation. Where possible, stool samples will be collected on site except for the “3 weeks before” and
9 215 “3 weeks after” treatment samples which the participant will collect at home. If a participant cannot
10 216 produce a stool sample during their clinical assessment visit, they will be given a stool collection kit to
11 217 take home. Collection kits contain a stool catcher, disposable gloves, specimen bag, specimen pottle,
12 218 DNA/RNA Shield Fecal Collection tube (#R1101, Zymo Research, Irvine, California, USA), and a step-
13 219 by-step instruction card. If the sample is collected at home, participants will be asked to store their
14 220 samples within their home freezer until their next appointment or arrange for collection by a member of
15 221 the research team. Upon receipt, stool samples will be aliquoted and stored in -80°C freezers at Te Ira
16 222 Kāwai – Auckland Regional Biobank. Stool collected in the DNA/RNA Shield Fecal Collection tube will be
17 223 reserved for gut microbiome assessment. Stool collected in the specimen pottle (i.e. not containing any
18 224 stabilisation buffer) will be reserved for metabolomics and intestinal inflammation assays (e.g.
19 225 calprotectin, lactoferrin, and S100A12 [62]).
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28 227 *Gut microbiome profiling*

29 228 DNA and RNA will be extracted using the ZymoBIOMICS MagBead DNA/RNA kit (Zymo Research,
30 229 #R2136) according to the manufacturer’s instructions with the addition of a bead bashing lysis step (Zymo
31 230 Research, #S6002-96-3). Shotgun metagenomic and metatranscriptomic sequencing will be performed
32 231 by a commercial provider using Illumina’s paired-end sequencing technology. Sequencing data will be
33 232 processed as performed previously [56], using bioBakery tools for meta’omic profiling [63]. In particular,
34 233 StrainPhlAn [64] will be used to generate single nucleotide polymorphism (SNP) haplotypes representing
35 234 the dominant strain of any given species within a sample. We will use these SNP haplotypes to compare
36 235 the genetic similarity of donor and recipient strains before and after treatment to assess the proportion
37 236 and stability of donor strain engraftment.
38
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43
44 238 *Blood sample collection*

45 239 Blood samples will be collected at baseline, 6 weeks, and 12 weeks after treatment to assess nutritional
46 240 status, inflammation, and liver/thyroid function (Table 4). A subset of these tests will be performed in real-
47 241 time throughout the study period for safety monitoring. These tests will also be repeated at the completion
48 242 of study for evaluation of study outcomes, avoiding any potential batch effects.
49
50
51 243

52 244 **Table 4.** Blood test schedule for all clinical assessment visits (baseline, week 6, week 12).

	Safety monitoring	Study outcomes
Electrolytes (Sodium, Potassium)	✓	✓
Creatinine	✓	✓
Ferritin	✓	✓
Total protein	✓	✓
Albumin	✓	✓

Alkaline phosphatase (ALP)	✓	✓
Alanine aminotransferase (ALT)	✓	✓
Gamma-glutamyl transferase (GGT)	✓	✓
Aspartate aminotransferase (AST)		✓
C-reactive protein (CRP)		✓
Cholinesterase		✓
Folate		✓
Vitamin B12		✓
Cortisol		✓
Free thyroxine		✓
Thyroid stimulating hormone (TSH)		✓
Serotonin (5-HT)		✓

245

246 *Anthropometry and body composition*

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

256

257 *Questionnaires*

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

265

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

271

272 Participants will also complete three established health questionnaires at baseline, 6 weeks, and 12
 273 weeks after treatment: 1) Eating Disorder Examination Questionnaire (EDEQ v6.0) [66], 2) Patient Health
 274 Questionnaire 9 (PHQ-9) for symptoms of depression [58], and 3) General Anxiety Disorder 7-item scale
 275 (GAD-7) [67].

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4 277 **Safety monitoring**
5
6 278 By adopting strict selection criteria for donors, we will reduce the risk of infection via FMT by minimising
7 279 the potential transmission of pathogenic organisms. Participants will take each dose of FMT in our clinic
8 280 under the supervision of a research clinician and/or nurse, where they will remain under close monitoring
9 281 for at least one hour afterwards. Based on our previous experience and existing evidence, it is unlikely
10 282 that participants will experience any severe adverse events [42]. However, participants will be instructed
11 283 to seek immediate medical attention if they develop any severe adverse reactions following treatment.
12 284 We will contact participants 24 hours after ingestion of each set of capsules, as well as 1, 3, 6, and 12
13 285 weeks after treatment to enquire about any adverse side effects. Specifically, participants will be asked
14 286 to report on the following events: loose or bloody stools, abdominal pain, vomiting, nausea, constipation,
15 287 flatulence, bloating, fever, malodorous burps, flu-like symptoms, allergic symptoms, appetite, fatigue, and
16 288 agitation. Adverse events will be graded in accordance with Common Terminology Criteria for Adverse
17 289 Events v4.0 (CTCAE) [68].
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24 291 In addition, we will monitor blood markers of nutritional status and liver function (Table 4), any available
25 292 body weight records as provided by the participant's clinical care team, and questionnaire scores
26 293 throughout the study in case any of the participants' health starts deteriorating. If the participant answers
27 294 "several days", "more than half the days" or "nearly every day" to PHQ-9, question 9 "Thoughts that you
28 295 would be better off dead or of hurting yourself in some way", the research clinician will interview the
29 296 participant further and provide them with safety management information to take home. Before the
30 297 participant leaves the clinic, the research clinician will also recommend clinical follow-up and contact the
31 298 participant's routine care provider and/or the research psychiatrist to ensure additional mental health
32 299 support is provided.
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39 301 These clinical and adverse event data will be reviewed by an independent data monitoring committee
40 302 (DMC) who can decide to stop the trial if the safety of participants is thought to have been compromised.
41 303 Any serious adverse event or clinical result will be notified immediately to the DMC.
42
43
44

45 305 We will strongly advise participants to bring a support person to their study appointments. The support
46 306 person could be a family member, friend, or member of their support team. Following discussions with
47 307 our advisers, this person would support the participant during and after the clinic visits, provide comfort
48 308 and reassurance to the participant throughout the study, and act as an additional point of contact in case
49 309 the participant becomes uncontactable during the study period.
50
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53 311 **Outcomes**

54 312 *Primary outcome*

- 55 313
- 56 314 • A shift in gut microbiome composition at 3 weeks post-FMT (Bray-Curtis dissimilarity). The shift
57 315 should exceed the drift in gut microbiome composition measured over the 3 weeks between
58 enrolment and baseline.

- 1
2
3 316
4 317 *Secondary outcomes*
5
6 318
 - Adverse events associated with FMT treatment

7 319
 - Proportion of participants who swallow all 20 treatment capsules

8
9 320
 - Proportion of participants who would consider having the treatment again if effective

10 321
 - Gut microbiome diversity, composition and functional potential at 6, and 12 weeks post-FMT

11 322
 - Donor strain engraftment at 3, 6, and 12 weeks post-FMT

12 323
 - Intestinal inflammation at 6 and 12 weeks post-FMT

13 324
 - Blood markers of nutritional status and liver/thyroid function at 6 and 12 weeks post-FMT

14 325
 - Eating disorder symptoms at 6 and 12 weeks post-FMT

15 326
 - Depression symptoms at 6 and 12 weeks post-FMT

16 327
 - Anxiety symptoms at 6 and 12 weeks post-FMT

17 328
 - BMI at 12 weeks post-FMT

18 329
 - Body composition at 12 weeks post-FMT

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331 **Sample size calculation**

332 Our primary outcome is a shift in the gut microbiome composition at 3 weeks post-FMT. Because we do
333 not have a control group to compare against, we will instead collect a stool sample 3 weeks prior to FMT
334 to assess the background drift in the gut microbiome over a 3 week period without any intervention. We
335 will use the Bray-Curtis dissimilarity index to compare gut microbiome composition profiles between
336 sampling time points and test for a difference in these values using a paired t-test. To identify a shift in
337 the gut microbiome above background drift, we will need 18 participants (80% power, alpha = 0.05). This
338 calculation was based on data from our previous FMT trial [42] comparing the gut microbiome shifts
339 between 39 FMT and 44 placebo recipients over 6 weeks (Bray-Curtis dissimilarity to baseline; FMT
340 mean 0.574, Placebo mean 0.416, delta = 0.158, SD = 0.163, t-test p = 2.754e-06). To account for a
341 potential dropout rate of 10%, we aim to recruit at least 20 participants who complete treatment and the
342 primary outcome assessment.
343

344 **Statistical analyses**

345 We will perform both intention-to-treat and per-protocol analyses. Per-protocol analyses will only include
346 data from those that complete the full treatment dose. Baseline demographics and clinical characteristics
347 will be summarised using descriptive statistics. Gut microbiome shifts will be assessed by calculating the
348 Bray-Curtis dissimilarity index to and from baseline using species-level relative abundance profiles. To
349 assess the primary outcome, a two-sided paired t-test will compare the potential shift in gut microbiome
350 composition 3 weeks before treatment to the shift 3 weeks after treatment. No imputation will be
351 performed for missing data, and statistical significance will be set at $p < 0.05$.
352

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

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3 356
4 357 Changes in clinical outcomes from baseline will be assessed using paired t-tests (parametric) or Wilcoxon
5 358 signed rank tests (non-parametric), as appropriate. However, we acknowledge that we cannot make any
6 359 treatment efficacy claims based on these paired within-group analyses and without a control group.
7
8 360

361 **Patient and public involvement**

362 This study has been co-designed in consultation with members from the Eating Disorders Association
363 New Zealand (EDANZ) as well as women who have previously recovered from AN. These discussions
364 ensured the study was designed appropriately to minimise participant stress and burden. The study
365 protocol, participant information sheet, and recruitment material have all been reviewed by EDANZ and
366 our study advisors. EDANZ has also offered to support in recruitment for the study by posting on their
367 social media platforms and recommending local clinics and services for us to contact.
368

369 **ETHICS AND DISSEMINATION**

370 **Ethics approval**

371 Ethics approval for the study was granted by the Central Health and Disability Ethics Committee
372 (reference number: 21/CEN/212). The study protocol adheres to the ethical guidelines outlined in the
373 Declaration of Helsinki [69]. All participants will provide written informed consent before participating in
374 the study.
375

376 **Data management**

377 Each participant in the study will be given a unique de-identified study ID that will be used to label all their
378 data and samples collected throughout the study. All recorded clinical data will be entered and stored in
379 the web-based platform REDCap, which is hosted in secure servers at the University of Auckland. Access
380 to these data will be restricted to the members of the research team. Clinical data will be stored for a
381 minimum period of 10 years. Biological samples (i.e., stool and blood samples) will be securely stored for
382 up to five years in -80°C freezers at Te Ira Kāwai - Auckland Regional Biobank, with access restricted to
383 members of the research team for the purposes of this study only. All study personnel involved in data
384 and tissue collection will be trained in good clinical practice (GCP), study protocol, and collection
385 requirements. Participants will have the right to access and correct their personal data without being
386 withdrawn from the study. If a participant withdraws from the study, any samples or data collected prior
387 to withdrawal will continue to be used and included in the study.
388

389 **Data availability**

390 At the completion of the study, the de-identified post-filtered metagenomic sequencing data will be made
391 publicly available on NCBI's sequence read archive (SRA). Note that this data set does not contain human
392 DNA sequences. The de-identified clinical data may be made available for future research upon valid
393 requests to the Liggins Institute Clinical Data Research Hub Data Access Committee. Requestors will
394 need to provide a methodologically sound proposal, obtain appropriate ethics approval, and sign a Data
395 Access Agreement. The Data Access Agreement will include a commitment to using the data only for the

396 specified proposal, not to attempt to identify any individual participants, to securely store and use the
397 data, and to destroy or return the data after completion of the project. Information on data sharing will be
398 provided in the participant information sheet and will be listed in the consent form.

399

400 **Dissemination**

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

406

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414

415 **AUTHOR CONTRIBUTIONS**

416 Funding acquisition: WC, JO

417 Consultation: BW, JD, BA, KL, CC, MD, HT, WC, JO

418 Study design: BW, JD, BA, KL, RTC, CC, MD, TE, TV, HT, WC, JO

419 Ethics application: BW, JD, BA, KL, TE, TV, HT, WC, JO

420 Protocol drafting: BW

421 Protocol revision: BW, JD, BA, KL, RTC, CC, MD, TE, TV, HT, WC, JO

422

423 **CONFLICTS OF INTERESTS**

424 The authors have no conflicts of interest to declare.

425

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429 data, or in the writing or decision to publish the manuscript on study findings.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 14

1	Roles and	#5b	Name and contact information for the trial sponsor	1
2	responsibilities:			
3	sponsor contact			
4	information			
5				
6				
7				
8	Roles and	#5c	Role of study sponsor and funders, if any, in study	14
9	responsibilities:		design; collection, management, analysis, and	
10	sponsor and funder		interpretation of data; writing of the report; and the	
11			decision to submit the report for publication, including	
12			whether they will have ultimate authority over any of	
13			these activities	
14				
15				
16				
17	Roles and	#5d	Composition, roles, and responsibilities of the	10
18	responsibilities:		coordinating centre, steering committee, endpoint	
19	committees		adjudication committee, data management team, and	
20			other individuals or groups overseeing the trial, if	
21			applicable (see Item 21a for data monitoring committee)	
22				
23				
24				
25				
26	Introduction			
27				
28	Background and	#6a	Description of research question and justification for	3,4
29	rationale		undertaking the trial, including summary of relevant	
30			studies (published and unpublished) examining benefits	
31			and harms for each intervention	
32				
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34				
35	Background and	#6b	Explanation for choice of comparators	11
36	rationale: choice of			
37	comparators			
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40	Objectives	#7	Specific objectives or hypotheses	11
41				
42				
43	Trial design	#8	Description of trial design including type of trial (eg,	4
44			parallel group, crossover, factorial, single group),	
45			allocation ratio, and framework (eg, superiority,	
46			equivalence, non-inferiority, exploratory)	
47				
48				
49	Methods:			
50	Participants,			
51	interventions, and			
52	outcomes			
53				
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56	Study setting	#9	Description of study settings (eg, community clinic,	4
57			academic hospital) and list of countries where data will	
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1			be collected. Reference to where list of study sites can	
2			be obtained	
3				
4	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	5
5			applicable, eligibility criteria for study centres and	
6			individuals who will perform the interventions (eg,	
7			surgeons, psychotherapists)	
8				
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10				
11	Interventions:	#11a	Interventions for each group with sufficient detail to allow	6,7
12	description		replication, including how and when they will be	
13			administered	
14				
15				
16	Interventions:	#11b	Criteria for discontinuing or modifying allocated	7, 10
17	modifications		interventions for a given trial participant (eg, drug dose	
18			change in response to harms, participant request, or	
19			improving / worsening disease)	
20				
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22				
23	Interventions:	#11c	Strategies to improve adherence to intervention	7, 10
24	adherence		protocols, and any procedures for monitoring adherence	
25			(eg, drug tablet return; laboratory tests)	
26				
27				
28	Interventions:	#11d	Relevant concomitant care and interventions that are	5
29	concomitant care		permitted or prohibited during the trial	
30				
31				
32	Outcomes	#12	Primary, secondary, and other outcomes, including the	11
33			specific measurement variable (eg, systolic blood	
34			pressure), analysis metric (eg, change from baseline,	
35			final value, time to event), method of aggregation (eg,	
36			median, proportion), and time point for each outcome.	
37			Explanation of the clinical relevance of chosen efficacy	
38			and harm outcomes is strongly recommended	
39				
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43	Participant timeline	#13	Time schedule of enrolment, interventions (including any	8
44			run-ins and washouts), assessments, and visits for	
45			participants. A schematic diagram is highly	
46			recommended (see Figure)	
47				
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49				
50	Sample size	#14	Estimated number of participants needed to achieve	11
51			study objectives and how it was determined, including	
52			clinical and statistical assumptions supporting any	
53			sample size calculations	
54				
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57	Recruitment	#15	Strategies for achieving adequate participant enrolment	4,6
58			to reach target sample size	
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60				

1 **Methods:**

2 **Assignment of**
3 **interventions (for**
4 **controlled trials)**
5
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8	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,
9	generation		computer-generated random numbers), and list of any
10			factors for stratification. To reduce predictability of a
11			random sequence, details of any planned restriction (eg,
12			blocking) should be provided in a separate document
13			that is unavailable to those who enrol participants or
14			assign interventions
15			
16			
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18			
19	Allocation	#16b	Mechanism of implementing the allocation sequence (eg,
20	concealment		central telephone; sequentially numbered, opaque,
21	mechanism		sealed envelopes), describing any steps to conceal the
22			sequence until interventions are assigned
23			
24			
25			
26	Allocation:	#16c	Who will generate the allocation sequence, who will
27	implementation		enrol participants, and who will assign participants to
28			interventions
29			
30			
31	Blinding (masking)	#17a	Who will be blinded after assignment to interventions
32			(eg, trial participants, care providers, outcome
33			assessors, data analysts), and how
34			
35			
36	Blinding (masking):	#17b	If blinded, circumstances under which unblinding is
37	emergency unblinding		permissible, and procedure for revealing a participant's
38			allocated intervention during the trial
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41 **Methods: Data**
42 **collection,**
43 **management, and**
44 **analysis**
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46
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48	Data collection plan	#18a	Plans for assessment and collection of outcome,
49			baseline, and other trial data, including any related
50			processes to promote data quality (eg, duplicate
51			measurements, training of assessors) and a description
52			of study instruments (eg, questionnaires, laboratory
53			tests) along with their reliability and validity, if known.
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Reference to where data collection forms can be found, if not in the protocol

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4	Data collection plan:	#18b	Plans to promote participant retention and complete
5	retention		follow-up, including list of any outcome data to be
6			collected for participants who discontinue or deviate from
7			intervention protocols
8			
9			
10	Data management	#19	Plans for data entry, coding, security, and storage,
11			including any related processes to promote data quality
12			(eg, double data entry; range checks for data values).
13			Reference to where details of data management
14			procedures can be found, if not in the protocol
15			
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19	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary
20			outcomes. Reference to where other details of the
21			statistical analysis plan can be found, if not in the
22			protocol
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26	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and
27	analyses		adjusted analyses)
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29			
30	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-
31	population and		adherence (eg, as randomised analysis), and any
32	missing data		statistical methods to handle missing data (eg, multiple
33			imputation)
34			
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36	Methods: Monitoring		
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39	Data monitoring:	#21a	Composition of data monitoring committee (DMC);
40	formal committee		summary of its role and reporting structure; statement of
41			whether it is independent from the sponsor and
42			competing interests; and reference to where further
43			details about its charter can be found, if not in the
44			protocol. Alternatively, an explanation of why a DMC is
45			not needed
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50	Data monitoring:	#21b	Description of any interim analyses and stopping
51	interim analysis		guidelines, including who will have access to these
52			interim results and make the final decision to terminate
53			the trial
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57	Harms	#22	Plans for collecting, assessing, reporting, and managing
58			solicited and spontaneously reported adverse events
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and other unintended effects of trial interventions or trial conduct

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4	Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
5			n/a
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9	Ethics and		
10	dissemination		
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13	Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval
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17	Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)
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25	Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
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30	Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
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36	Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
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43	Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site
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47	Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
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52	Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
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57	Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the
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public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions

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5	Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of
6	authorship		professional writers
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9	Dissemination policy:	#31c	Plans, if any, for granting public access to the full
10	reproducible research		protocol, participant-level dataset, and statistical code
11			
12			

13 Appendices

14			
15	Informed consent	#32	Model consent form and other related documentation
16	materials		given to participants and authorised surrogates
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19	Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of
20			biological specimens for genetic or molecular analysis in
21			the current trial and for future use in ancillary studies, if
22			applicable
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 28 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
 29 [Penelope.ai](#)
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