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Randomised, placebo-controlled, double-blinded trial of fecal microbiota transplantation in severe obesity: a study protocol

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ABSTRACT

Introduction Obesity is one of the main threats to public health in western countries and increases the risk of several diseases, overall morbidity and mortality. Sustained weight loss will reduce risk factors and improve several obesity comorbidities. Options are conservative treatment such as lifestyle changes, bariatric surgery or medications. Conservative treatment has a low success rate, and bariatric surgery is typically not reversible, with the risk of complications and recurrences. Treatment of obesity with medications has in recent years shown great promise, but the side effects are many, and the long-term effect is unknown. There is also a need for an option for patients where surgery has contraindications and conservative follow-up does not succeed.

The research on obesity and gut microbiota has yielded promising results regarding weight reduction and metabolic health, but more research is needed to better understand the relationship between gut microbiota and severe obesity. This study could show proof of concept that gut microbiota from a lean donor could, in addition to lifestyle intervention, contribute to weight reduction in people suffering from severe obesity.

Method and analysis This study aims to investigate if a fecal microbiota transplantation (FMT) from a lean donor leads to weight reduction in participants suffering from severe obesity. The study is a single-centre, double-blinded, placebo-controlled, parallel-group study with 60 participants. Participants will be randomised 1:1 for FMT from a lean donor or placebo. FMT or placebo will be delivered once by enema.

We will include participants from the outpatient clinic for severe obesity, at the Medical Department, University Hospital of North Norway, Harstad, by invitation only. The study has a follow-up period of 12 months, with study visits of 3, 6 and 12 months post FMT. The primary endpoint is a weight reduction of ≥10%, 12 months after intervention.

The results of the study will be published in open access journals. At the end of the study, the participants will receive information on which treatment group they belong to.

Ethics and dissemination The Regional Ethical Committee in North Norway (REK) approved the study protocol (2017/1655/REK Nord). We plan to present the results from the study at (inter)national conferences and publish in open-access general peer-reviewed journals. The enema method for FMT administration used in this study was developed by our study team.

Trial registration number NCT03273855.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study is relatively small but may serve as a proof of concept before launching a large-scale study.
⇒ The study has a strong design, being a randomised double-blinded controlled study, and a long follow-up period allows evaluation of the robustness of effect (if any).
⇒ The design only allows for conclusions on the effect of fecal microbiota transplantation (FMT) in obesity, when delivered by enema; other delivering methods for the FMT may show different results.
⇒ Extensive biobanking, questionnaires regarding childhood trauma, binge eating, food consumption, physical activity and perception of one's own health, allow us to look at severe obesity from several different angles.
⇒ Fecal samples collected in the study will undergo metagenome sequencing, which will give valuable information regarding both the functional potential and bacterial composition of the microbiome in severe obesity.

INTRODUCTION

The gut microbiota is recognised as an environmental modulator of nutritional uptake and body weight.[1] This has led to the hypothesis that the gut microbiota could be a therapeutic target for fighting obesity. Fecal microbiota transplantation (FMT) has been applied for more than 50 years and is an established treatment for recurrent infection with *Clostridioides difficile*.[2] Recent studies have shown that alterations in the composition of the gut microbiota could be the cause of several diseases.[3 4] If the balance between the

host and microbiota is disturbed, pathological conditions may arise in both the gastrointestinal, neurological and respiratory system.[5]

A 2014 article by Ridaura *et al* showed that obesity-associated metabolic phenotypes, total body mass and fat mass, was transferable from obesity-incongruent twins to germ-free mice. When mice that received gut microbiota from the lean twin (Ln) were cohoused with mice that received microbiota from the obese twin (Ob), the Ob mice were protected from developing the obesity-related metabolic phenotypes and weight gain. It seemed that certain members of the genus Bacteriodites were transmitted from Ln mice to Ob mice through the diet and was responsible for the protection. This suggests that there is an interaction between diet and microbiota and that the effect could be transferred and modified.[6]

The diet and host digestion of carbohydrates affect the amount of end-product short-chain fatty acid (SCFA) in the stool. SCFA has shown beneficial effects on the host metabolism, normalising glucose level and reducing plasma levels of cholesterol.[7] Studies on mice have shown that increased production of SCFA by decreased Bacteroidetes and increased Firmicutes can promote obesity by increasing colonic energy availability.[8] A difference in gut bacterial composition between people with severe obesity and normal weight has been shown in several studies.[9 10] The footprint shown is reduced microbial diversity and gene richness in people suffering from severe obesity. Data are conflicting, but most studies show a higher number of bacteria from the Firmicutes, Proteobacteria, *Lactobacillus* and Fusobacteria in individuals with obesity and a lower number of Bacteroidetes, *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*. An increase in the Firmicutes/Bacteroidetes ratio is associated with obesity.[9]

People suffering from severe obesity have a systemic low-grade inflammation. In a study of 15 obese women (body mass index >32), Maachi *et al* showed a strong association between inflammatory markers and adipocytokines. The systemic low-grade inflammation was associated with elevated levels of tumour necrosis factor-α, interleukin-6 and leptin, in both circulating and adipose tissue, and elevated levels of serum C-reactive protein. These results suggest that people suffering from obesity have increased inflammatory proteins secreted by the liver, which could be influensed by cytokines secreted by adipose tissue.[11]

Our study will use high-sensitive C reactive protein (hs-CRP) as a screening test for this low-grade inflammation. A complete cytokine analysis will give further depth information on the systemic low-grade inflammation and provide information to what extent we find agreement between hs-CRP and a proinflammatory change in the cytokines and how this develops between the groups.

Hypothesis and objectives

We hypothesise that transferring gut microbiota from a lean and healthy donor to patients with severe obesity will contribute to weight reduction by a change in the gut microbiota of the recipients.

This project aims to determine if there is a relationship between a dysbiotic gut flora and obesity, by testing if a change of colonic microbiota through FMT can lead to weight reduction of ≥10%. The collection of biological materials both before and after intervention allows further analysis to elucidate the role of the gut microbiota in obesity, energy metabolism and immune response.

METHOD

Trial design

This is a single-centre, double-blinded, placebo-controlled, parallel-group study with a 12-month follow-up, performed at the Medical Department of the University Hospital of North Norway in the city of Harstad (UNN, Harstad). The Regional Ethical Committee in North Norway (REK) approved the study in March 2019. The study will be performed in accordance with the Helsinki Declaration.

Participants will be recruited from the outpatient clinic for severe obesity at invitation only, according to predetermined inclusion and exclusion criteria (table 1). Patients undergo a physical examination, laboratory workup and medical history before inclusion in the study. Eligible participants provide fecal and blood samples for biobanking and measurement of heart rate variability (HRV) and answer patient-reported outcome measurements (PROMs) before they are allocated to either placebo or active FMT treatment. The follow-up period lasts for 12 months, with checkpoints 1, 3, 6 and 12 months after treatment (figures 1 and 2).

Patient and public involvement statement

Our outpatient clinic for severe obesity has a tradition for user involvement and participation from the establishment of the outpatient clinic. Our active patient representative was involved in designing the project with a meeting before inclusion and also planned meetings in all project phases. Additionally, we had a dialogue with the patient organisation, the national association of obesity sufferers. The representatives describe that in their knowledge, this intervention will be of interest in the patient group, and they believe that recruitment is possible.

Study population

Study participants

We plan to include 60 participants, recruited consecutively from our outpatient clinic for patients with severe obesity at the Medical Department, UNN Harstad. At the outpatient clinic, all the participants will undergo a 12-month lifestyle change programme while participating in the study. Patients will be asked to participate in the study if they fulfil the study criteria listed in table 1. A study personnel will make an appointment with potential study participants after their first visit at the outpatient clinic, for evaluation of inclusion criteria, give information and obtain informed consent if they have expressed an interest in participating in the study. Approximately

Table 1 Inclusion and exclusion criteria for participation and fecal donors in the randomised controlled trial of fecal microbiota transplantation in severe obesity

Inclusion	Exclusion
Study participants	
Must be at least 18 years of age and under 69 years	Symptomatic cardiovascular disease, lung disease, cirrhosis or significant renal failure
Sign consent form	Patients who are pregnant or breast feeding
BMI ≥ 40 kg/m^2 or BMI ≥ 35 kg/m^2 combined with comorbidity related to obesity	Patients who have a confirmed malignancy or cancer
	Patients who are immunocompromised
	Previous gastric or small intestinal surgery that alters gut anatomy such as fundoplication, gastric resection, gastric bypass or small bowel resection
	Established drug or alcohol abuse or particularly unstable psychosocial circumstances
	History of cholecystectomy
	New drugs the last 3 months or during the follow-up period that can impact on metabolism or body weight
	Antibiotic treatment the last 3 months
	Serious food allergies
Fecal donors	
Age 16–40	Use of peroral antibiotics the past 6 months
Sign consent form	Tattoo or piercing the past 6 months
Healthy	Close relatives with serious autoimmune disease, psychiatric disorder or obesity
BMI 18–25	Former imprisonment
	History of Chronic diarrhoea Constipation Inflammatory bowel disease Irritable bowel syndrome Colorectal polyps Immunosuppression Obesity Metabolic syndrome CFS/ME Psychiatric disorders Other serious autoimmune diseases Cancer
	High-risk sexual behaviour
	Bowel movements that do not correspond to a Bristol Stool Scale type 3 or 4
	Journeys abroad the last 6 months to countries high in antibiotic resistance
	Use of food supplements, prebiotics, probiotics or symbiotics
	Positive cultures for extended spectrum beta-lactamase (ESBL), VRE and MRSA, *Salmonella* spp, *Shigella* spp, *Campylobacter* spp,*Yersinia* spp and toxin-producing *Clostridioides difficile*. Fecal tests for viruses (norovirus, rotavirus, sapovirus and adenovirus) and occult blood and a GA-map dysbiosis test: with dysbiosis grade 3 or more*[17].
	Blood samples for glycated haemoglobin, serology for HIV, *Treponema pallidum* and hepatitis A, B, C and E

*The bacteria measured with the GA-map dysbiosis test do not represent entire phyla, but only specific parts of the phyla. This is a clear limitation of the test, which the study personnel is aware of.
BMI, body mass index; CFS, chronic fatigue syndrome; ESBL, extended spectrum beta-lactamase; ME, myalgic encephalomyelitis; MRSA, methicillin-resistant Staphylococcus aureus; VRE, vancomycin-resistant enterococci.

100 new patients are referred to our obesity outpatient clinic each year.

Donors

Only individuals matching the criteria in table 1 and the European consensus criteria on FMT in clinical practice[12] are eligible for recruitment as fecal donors. The recruitment of donors will be from the local community and high schools. The study plans to use 2 donors, treating 15 participants with donor A and 15 participants with donor B. The complete screening will be undertaken at the first fecal delivery and every 8th week, as long as the donors are active in the study. The inclusion and screening will be performed at the Medical Department of UNN, Harstad. We will record every reason for failure during the recruitment process.

Due to difficulties in the inclusion process, the storage time of the FMT transplants from the first two donors surpassed 24 months and it was decided to include two

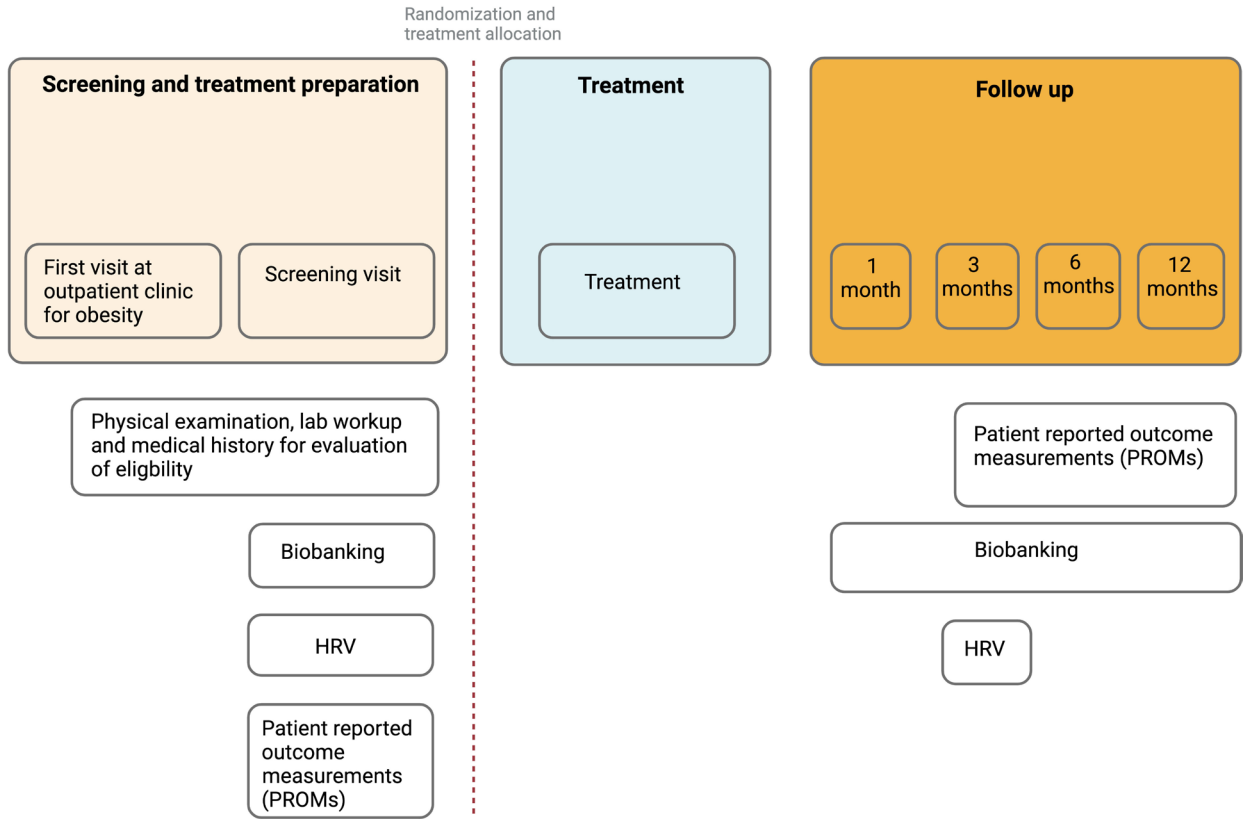

Figure 1 Patient flow and data capture, redrawn from C.O.L.O.N.I.Z.E/Frederik Emil Juul, created with BioRender.com.

new donors, meaning that the study ended up using four donors.

Preparation of FMT

Fresh donated feces (50 g) are mixed with 25 mL 85% glycerol and 120 mL saline to a homogenised solution and poured through a 0.5 mm mesh strainer. The solution is transferred to four 50 mL Luer-Lok syringes. All FMT products are stored in the study's biobank at −80°C, with a unique identification tag:

Donor transplant: Date of donation+DonorID

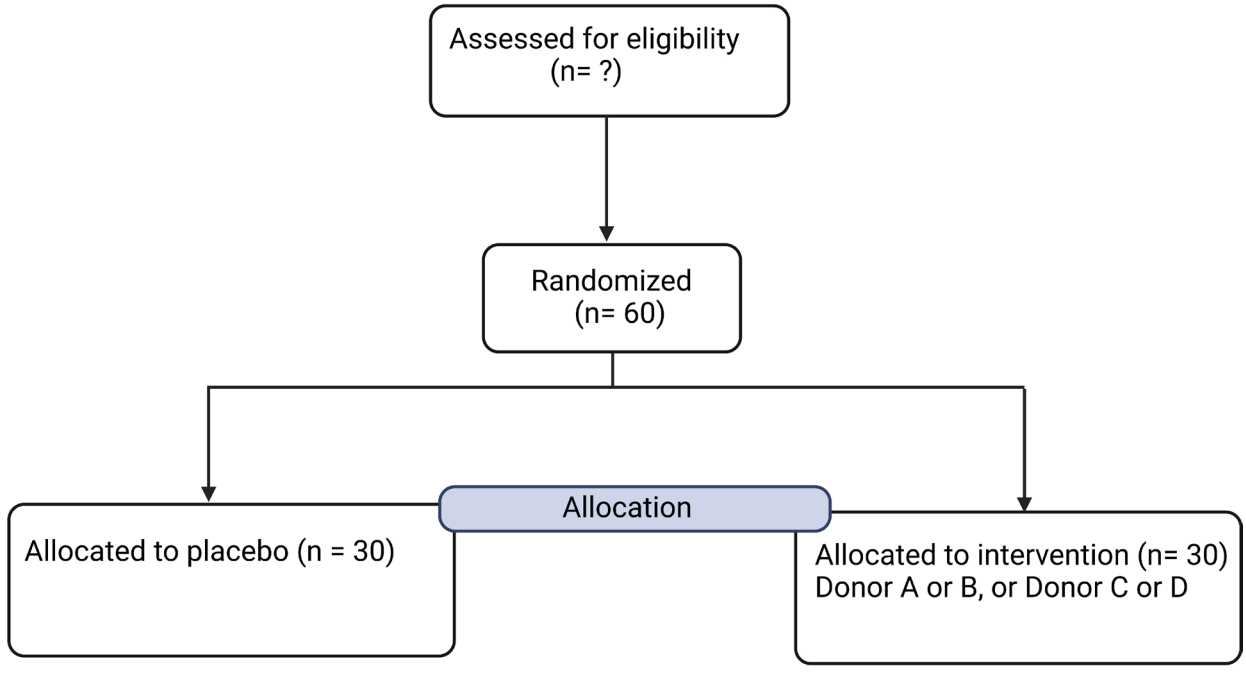

Figure 2 Study flow chart, created with BioRender.com.

Preparation of placebo

Study participants collect samples of their own feces at home and store them in their home freezer until transportation. All samples must be delivered frozen to UNN, Harstad, where they are further biobanked and stored at −80°C. Blood samples (full blood, plasma and serum) and fecal samples are obtained before treatment and 3, 6 and 12 months after treatment. Samples are stored in a general biobank for dysbiosis-related research (REK North 184045).

Placebo is prepared according to the same protocol as the active transplant. Placebo transplants are prepared during the inclusion process before intervention, and is the participants' own feces.

Intervention

Before administration, the frozen transplant is placed in a water bath at 37°C. The thawing lasts for 1 hour, then the transplant is transferred to an enema bag, and 240 mL isotonic saline is added before installation.

FMT procedure

Intervention will take place at the gastroenterology outpatient clinic at UNN, Harstad, Norway. No antibiotics will be given prior to the intervention.

The study will use enema for administrating the FMT, a procedure developed at the Medical Department of UNN, Harstad, by the research group.[13] The participants will perform a bowel lavage using sodium picosulfate/magnesium citrate (Picoprep, Ferring) 24 hours before the delivery of FMT and take 8 mg of loperamide 1 hour prior to the FMT procedure.

Procedure for administrating FMT

The participant will lie on his/her left side in Trendelenburg's position, while the examiner performs a digital examination. The probe from the enema kit is lubricated and inserted in the rectum before the rectal balloon inflated (to prevent leaking). The patient is further positioned to ensure a proximal colonic distribution of the FMT by the following procedure:

1. The participant lies on his/her left side with the bench tilted in Trendelenburg's position for 2 min.
2. The participant turns directly to an abdominal position and holds it for 2 min. The head and body should still be tilted down in Trendelenburg's position.
3. The participant turns slowly until lying on the right side and holds this position for 2 min.
4. The bench is then tilted the opposite way (anti-Trendelenburg), and the position is held for 2 min.
5. The balloon around the rectal probe is deflated and removed from the rectum, the bench still in anti-Trendelenburgs position. This position is held for 10 minutes. If the participant feels the urge to defecate, he/she should immediately be guided to a toilet to avoid soiling.

When getting up, the patient should go directly from the position lying on the right to a standing position. We will encourage the participant to keep the solution in the colon as long as possible. We will register the time from FMT treatment to defecation. After the intervention, the participants have no restrictions on activity level.

Outcomes

In this study, we will collect baseline variables such as demographic data (eg, age, gender, height, waist circumference and blood pressure) together with several PROMs, all listed in table 2.

Primary outcome

The primary outcome is to determine if there is a relationship between a dysbiotic gut flora and obesity, by testing if a change of colonic microbiota through FMT can lead to weight reduction of ≥10%, 12 months post FMT.

Secondary outcome

Secondary outcomes are listed in table 3.

Exploratory evaluation

In addition to the clinical effectiveness of FMT on obesity, the following research questions will be investigated.

1. Donor microbiota engraftment 1, 3, 6 and 12 months post FMT: A comparison between baseline profile, post FMT and donor profile will show if engraftment of donor microbiota parallels clinical response to active FMT.
2. Questionnaire regarding COVID-19 vaccine and infection: Due to the COVID-19 virus, participants included and in the follow-up period from June 21 will be asked two questions on COVID-19 vaccination and COVID-19 infection
3. We will explore the FMT effects on HRV and the vagal nerve. Comparing the participants who received placebo and active transplant will give valuable insight into whether participants who received active transplant have restored the equilibrium between the sympathetic and parasympathetic nervous system responsible for the maintenance of autonomic homeostasis

Determination of sample size

To determine the sample size, we looked at data from our outpatient clinic and found that patients have an average weight loss of 2.5% with our conservative treatment, SD near 7. This will therefore be the expected result in the control group (receiving placebo). A weight reduction of ≥10% leads to significant improvement in health and quality of life,[14] and a weight change of this magnitude is therefore considered clinically relevant. The difference between the two groups is estimated to be 7.5%, and with these historical results, the sample size is estimated to be 19 participants in each group with a power of 0.90 and significance level of 0.05. We will eliminate extreme values, more than 3 SD out of the average in the group. We must be prepared for a high drgree of loss to follow-up (near one third), as this is the experience from the outpatient clinic for severe obesity.[15] Therefore, we will include 60 participants, 30 in each group.

Table 2 Trial schedule with data capture of patient-reported outcomes

	Screening period	Treatment period	Follow-up period			
	First meeting/ inclusion	Treatment day	1 month	3 months	6 months	12 months
Informed consent and inclusion/ exclusion evaluation	X					
Physical examination and vital signs	X*			X*	X*	X*
15 min of continuous ECG for HRV measurement†	X‡			X‡		
Short Difficult Childhood Questionnaire	X					
Binge Eating Questionnaire	X					X
RAND-36	X					X
HSCL-25	X					X
IPAQ	X			X	X	
CTQ	X					
Questions about COVID-19 vaccination and infection§	X		X	X	X	X
Questions regarding the lockdown due to COVID-19¶	X		X	X	X	X
Patient-Reported Adverse Event				X	X	X
FFQ	X			X	X	X
iDXA total body	X					X
Blood samples	X**††			X††	X††	X††
Fecal sample	X‡‡§§		X§§	X§§	X§§	X§§
Fecal microbiota transplantation		X				

Data capture in the electronic case report form. Data capture on paper.
*Blood pressure, pulse, weight loss in per cent and anthropometric measurement.
†The HRV measurement will only be performed on the last 20 participants in the study.
‡Will be measured by the participant at home as instructed by the study personnel.
§Included participants, and participants still in the follow-up period, were asked questions about COVID-19 vaccination and COVID-19 infection from June 21.
¶Will only be given to participants who were in the follow-up period from March 20 to July 20.
**Haemoglobin (Hb), leucocytes, thrombocytes, folate, iron, ferritin, total iron-binding capacity, vitamin B_{12} and vitamin D.
††High-sensitive C reactive protein, erythrocyte sedimentation rate, Hb, hematocrit, white blood cell count, platelets, sodium, potassium, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, low-density lipoprotein, high-density lipoprotein (including subgroups), triglycerides, thyroid-stimulating hormone, free thyroxine 4, HbA1c, C-peptide, cholesterol, amylase, fasting glucose level, insulin, quantifying the sensitivity and beta-cell function (Homeostatic Model Assessment of Insulin Resistance and Homeostatic Model Assessment of beta cell function) using calculators, cytokines, Tempus RNA for storage.
‡‡Short-chain fatty acid (SCFA), microbiota, possible placebo and storage.
§§SCFA, microbiota and storage.
CTQ, Childhood Trauma Questionnaire; FFQ, Food Frequency Questionnaire; HRV, heart rate variability; HSCL-25, Hopkins Symptoms Checklist-25; iDXA, dual-energy X-ray absorptiometry; IPAQ, International Physical Activity Questionnaire.

Randomisation and blinding

A research nurse at the Department of Clinical Research at UNN, Harstad, creates the allocation sequence using the REDCap software. The treatment is randomised in fixed blocks of four with two active (one donor A and one donor B or one donor C and one donor D) and two placebo.

Allocation—procedure to randomise participants

The allocator uses the randomisation sequence in the REDCap software to allocate active transplant or placebo to the participants and is the only personnel involved in the study that has access to this part of the software. Participants randomised to active treatment will have their tag on the placebo transplants switched to the donor transplant by the allocator on allocation. The placebo transplant will be disposed of immediately in the same process. All allocated transplants are placed in a designated box in the study freezer, ready for transplantation.

The allocator is responsible for establishing a paper key file, matching the study ID with the allocated treatment. This file is to be kept in a locked safe and scanned into a computer on the hospital's server. The file will allow

Table 3 Secondary endpoints, objectives and assessments

Objectives	Endpoints	Assessments
Secondary		
Weight reduction	Changes in % body weight	We will report participants who have a weight loss of ≥5%, >15% and 20%, using chi-square
Waist circumference		Measured in centimetres at inclusion and all follow-up visits
Gut microbiota composition and function		Identify bacterial flora using metagenome sequencing[16]
Lipid profile will be analysed in blood samples taken 3, 6 and 12 months post FMT		Cholesterol, LDL, HDL including subgroups, triglycerides (collaboration with Nordlands Hospital in Bodø)
A cytokine panel will be analysed in blood samples taken 3, 6 and 12 months post FMT		27 different cytokines (TNF-a, IFN-g, IL-1b, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, MCP1, IP-10, eotaxin, MIP-1a, MIP-1b, RANTES, G-CSF, GM-CSF, basic FGF, PDGF-BB and VEGF)
Content of short-chain fatty acid in faeces will be analysed in fecal samples taken 1, 3, 6 and 12 months post FMT		Measurement of SCFA in faeces (collaboration with Lovisenberg Diaconal Hospital, Oslo)
Insulin resistance will be measured at inclusion, 3, 6 and 12 months post FMT		s-Glucose, HbA1c, C peptide, insulin and fasting glucose levels
Blood pressure will be measured at inclusion, 3, 6 and 12 months post FMT		Collected as the average of the last two out of three measurements, at the end of 5 min resting period in supine position
Inflammation will be evaluated using blood sample analysis		hs-CRP, erythrocyte sedimentation rate, IL-6
Biochemical parameters of hepatic steatosis will be evaluated using blood sample analysis		ASAT, ALAT, ALP, ɣGT and amylase
Quality of life will be evaluated using RAND-36 questionnaire		Short-Form Health Survey Questionnaire (RAND-36): quality of life will be assessed by RAND-36, which is a validated instrument for general quality of life.[18] A score of 100 is equivalent to perfect health
Traumatic childhood and mental health disorder, share, correlation and relation to weight gain		Hopkins Symptoms Checklist-25: measures general psychological distress such as anxiety and depression[19]. A score of 1.75 or above indicated a clinically relevant level of symptoms of depression or anxiety Binge eating scale: subjective self-reporting on binge eating symptom pressure. Non-binge, less than 17; moderate binge, 18–26; severe binge, 27 and greater Short Difficult Childhood Questionnaire: measures whether the study participants have experienced difficulties in their childhood, by non-intrusive items including subjective evaluations of their childhood.[20] The questionnaire has a maximal score of 20 Childhood Trauma Questionnaire: assesses a broad range of traumatic experiences in childhood. The questionnaire allows us to evaluate emotional, physical and sexual abuse, together with physical and emotional neglect.[21] The questionnaire will be scored in categories of physical abuse (≥8), emotional abuse (≥8), sexual abuse (≥6), physical neglect (≥8) and emotional neglect (≥10)
Heart rate variability		Heart rate variability will be measured using Firstbeat Lifestyle Assessment at inclusion and at 3-month follow-up. The measurement will be done at home by the study participants, as instructed by the study personnel. This will allow us to investigate if recovery of normal gut microbiota by treatment with FMT from a healthy donor restores the equilibrium between the sympathetic and parasympathetic nervous system responsible for the maintenance of autonomic homeostasis

Continued

Table 3 Continued

Objectives	Endpoints	Assessments
Patient-Reported Adverse Event Questionnaire		Questions regarding adverse events, allowing us to detect if any of the participants have any adverse reactions during the study period
International Physical Activity Questionnaire		Collects data on health-related physical activity[22]

ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; FGF, fibroblast growth factors; FMT, fecal microbiota transplantation; G-CSF, Granulocyte-Colony Stimulating Factor; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; GT, gamma-glutamyltransferase; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; hs-CRP, high-sensitive C reactive protein; IFN, interferon; IL, interleukin; IP-10, interferon gamma-induced protein 10; LDL, low-density lipoprotein; MCP1, monocyte chemoattractant protein-1 ; MIP, Macrophage Inflammatory Proteins; PDGF-BB, platelet-derived growth factor - two B subunits; SCFA, short-chain fatty acid; TNF, tumuor necrosis factor; VEGF, vascular endothelial growth factor.

for tracking of the individual donor batch to the corresponding treatment given to the study participants, when follow-up is completed. Only the allocator will have access to the paper and data file.

Blinding

The study is double blinded, meaning that neither participants, outcome assessors or investigators are involved in the allocation or intervention. A person, the allocator, will be responsible for allocating treatment to participants. The allocator will not know the identity of the study participants, only their study ID. He or she will not be involved in the screening, inclusion process, follow up, data handling or the treatment of the study participants. This will ensure that the allocator is kept blinded, and will be the only person that has access to the randomization module in the REDCap software.

If any adverse events (AEs) occur, the principal investigator (PCV) has the authority to emergency unblind the study. Should the study need to unblind for any reason, the details for doing so, and the outcome, will be documented in an adverse reaction report. It will be the principal investigators assessment, whether any study personnel or any of the participants need to be unblinded.

Statistical method and data analysis

Primary analysis

The primary analysis will be evaluated as intention to treat, where the last valid observation will be used where variables are missing.

The primary outcome measure, a weight reduction of ≥10% in the intervention group, will be presented as bar charts with a comparison between the intervention and control group. Chi-square or Fisher's exact test will be used to present responders and non-responders in the active and control group. We will use odds ratio to present responders in the active group.

Secondary analysis

Per-protocol analysis of the primary endpoint includes only the participants who completed the study.

We will do a two-side t-test (Student's t-test) comparing weight loss per cent between groups.

Weight change of ≥5%, >15% and >20% will be presented as bar charts, with comparison between the intervention and control group.

We will analyse metagenomic data in the pipeline described in earlier publication.[16]

Repeated measures, analysis of variance, will be used to measure the effect of FMT on RAND-36. Disease duration, donor and treatment group (placebo and active treatment) will be used as predictors. Non-significant terms will be removed.

We will explore the FMT effects on changes in cytokine profile. The measurements will be taken before FMT and 3, 6 and 12 months post FMT. A comparison between the cytokine panels will give valuable insight into whether FMT can contribute to reduce inflammatory cytokine response.

We will explore the FMT effects on changes in lipid profile before FMT and 3, 6 and 12 months post FMT. Comparing the lipid profile taken before FMT to the lipid profile after 3, 6 and 12 months post FMT will give valuable insight in whether FMT can contribute to changing the lipid profile.

We will investigate the SCFA content of fecal samples in our participants, both before and 3, 6 and 12 months post FMT. This will allow us to investigate if our obese participants all have elevated fecal SCFA content and whether this will change after the FMT.

We will explore the FMT effects on insulin resistance, by measuring fasting glucose, insulin, C peptide and haemoglobin A1c before FMT and 3, 6 and 12 months after FMT. The sensitivity and beta-cell function (Homeostatic Model Assessment of Insulin Resistance and Homeostatic Model Assessment of beta cell function) will be quantified in the same timeframe. This will give valuable insight into whether FMT and weight loss can contribute to reduction of metabolic disease.

Due to the COVID-19 virus, the participants will be asked five questions regarding the impact of the restrictions introduced by the government on lifestyle changes and eating habits.

Short Difficult Childhood Questionnaire, CTQ and HSCL-25 will be analysed as numbers with a positive score, share and contribution and correlation between

them. Furthermore, we will analyse to which degrees these parameters affect weight reduction at the end of follow-up.

The statistical analyses will be done by using SPSS and R.

Procedure for discontinuation

Criteria for participant discontinuation

Participants may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a participant for this study are as follows:

1. Voluntary discontinuation by the participant who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
2. Safety reason as judged by the PI.
3. Major protocol deviation.
4. Incorrect enrolment, that is, the participant does not meet the required inclusion/exclusion criteria for the study.
5. Participant lost to follow-up.
6. Pregnancy.
7. Participant's non-compliance to study treatment and/or procedures.

Participant discontinuation

Participants who withdraw or are withdrawn from the study will still undertake the intended regular follow-up at the outpatient clinic for severe obesity. The reasons for discontinuation will be recorded. Participants who withdraw or are withdrawn will not be replaced. All participants receiving treatment will be included in the intention-to-treat population.

Trial discontinuation

The whole trial may be discontinued at the discretion of the PI or the sponsor in the event of any of the following:

1. Occurrence of AE unknown to date in respect of their nature, severity and duration.
2. Medical or ethical reasons affecting the continued performance of the trial.
3. Difficulties in the recruitment of participants.

The sponsor and PIs will inform all investigators, the relevant competent authorities and ethics committees of the termination of the trial along with the reasons for such action.

Ethics and dissemination

Safety considerations

The study compares FMT treatment to placebo (participants' own gut flora). The fecal donors in the study have undergone an extensive screening process to ensure the safety of the fecal transplant.

The administration method for FMT is done by enema, and there have been few reported serious AEs (SAEs) using enema in FMT. The procedure is non-invasive, uses minimal recourses and is found to be safe. The study was approved by the Norwegian Regional Ethics Committee, 2017/1655/REK Nord.

Safety board

The safety board consists of PCV, BK, RG and HMH. Telematics and physical meetings will be arranged for an update on the project. If any AEs are reported, PCV and HMH evaluate and involve RG and BK if necessary. If any SAEs are reported, FMT will be stopped until the board has discussed further measures. Patient-reported AEs will be documented in a separate questionnaire. A suspect adverse reaction report will follow any suspicion of an AE. In addition to asking for patient-reported AEs at 3, 6 and 12 months post FMT, participants can reach one of the investigators at any time by the phone number indicated in the consent form. SAEs or symptom deterioration for participants will prompt evaluation for opening the randomisation sequence and premature termination of the study, and the board will arrange a telematic meeting promptly.

Data management

Participants will be given a unique study ID, which can only be connected to personal information by study personnel. The study ID is used for all documentation, reports and publication. Data are collected using REDCap software. Patient-reported outcomes are obtained in the electronic case report form, except for the food frequency questionnaire. All data are stored for 15 years, and the biological material is stored for 5 years in the study biobank.

Dissemination

When the study is complete, we plan to present the results at (inter)national congresses and submit the manuscript to general open access peer-relieved journals. Authorship is according to the International Committee of Medical Journal Editors guidelines.

End of study

The study ends when the last participant's last visit (last of study visit) is completed. In other words, when 60 participants are given treatment and have completed their 365-day follow-up or have withdrawn from the trial or if a trial discontinuation criterion is met.

Trial status

We have currently recruited all 60 participants and the last participant's last visit was in August 2023. The reason that the study protocol has not been sent for publication earlier is due to difficulties in participant recruitment and the COVID-19 pandemic. Most of the study team had to do clinical work during the pandemic, making it difficult to prepare the study protocol for publication.

Contributors PCV, MSF, PHJ, BK and RG have contributed to securing research funding. HMH, PCV, MSF, LS, PHJ, KHA and BK have been part of the finalisation of the study design and writing of the protocol. LS, PHJ and HMH planned and performed the donor screening programme and were responsible for the implementation of the programme. HMH is the PhD student in the project and has had the final review of the protocol. All authors critically revised the protocol, gave their final approval and agreed to be accountable for all aspects and ensure integrity and accuracy.

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Competing interests The authors have declared these conflicts of interest: PHJ: principal investigator in REFIT 2 trial. An investigator-initiated and investigator-run randomised controlled trial investigating fecal microbiota transplantation by enema in patients with irritable bowel syndrome. MSF: payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Novo Nordisk. HMH: subinvestigator in 'fecal microbiota transplantation in CFS/ME', member of scientific committee in REFIT2 trial and member of scientific committee in COLONIZE trial. KA: payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Novo Nordisk.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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