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Title page

Randomized, placebo-controlled, double-blinded trial of fecal microbiota transplantation in severe obesity: A Study protocol

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Abstract

Introduction:

Obesity is the main threat 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 co-morbidities. Options are conservative treatment such as life style changes, bariatric surgery or medications. Conservative treatment has a low success rate, and bariatric surgery is irreversible, 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 do 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 life style intervention, contribute to weight reduction in people suffering from severe obesity.

Method and analysis:

This study aims to investigate if a transfer of fecal microbiota (FMT) from a lean donor leads to weight reduction in participants suffering from severe obesity. The study is a single-center, double-blinded, placebo-controlled, parallel-group study with 60 participants. Participants will be randomized 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, Medical department, University Hospital North Norway, by invitation only. The study has a follow-up period of 12 months, with study visits 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 [1].

Trial registration number:

The trial is registered at clinicaltrials.gov with trial registration number NCT 03273855.

Strenghts and limitations:

- 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 randomized 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 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 allows us to look at severe obesity from several different angles.
- Fecal samples collected in the study will undergo whole genome sequencing, which will give valuable information regarding the both the functional potential and bacterial composition of the microbiome in severe obesity.

Keywords

Severe obesity, fecal microbiota transplantation, weight reduction, gut microbiota, lifestyle intervention.

Introduction

The gut microbiota is recognized as an environmental modulator of nutritional uptake and body weight [2]. 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* (CDI) [3]. Recent studies have shown that alterations in the composition of the gut microbiota could be the cause of several diseases [4, 5]. Pathological conditions in the gastrointestinal, nervous, and respiratory systems could occur if the equilibrium between the host and microbiota is disturbed [6].

A paper from 2014 by Ridaura. et.al showed that obesity-associated metabolic phenotypes, total body, and fat mass was transmissible from twins discordant for obesity to germ free mice. Co-housing mice who had received microbiota from the obese twin (Ob) with mice who had received the lean twin's microbiota (Ln) prevented development of obesity associated phenotypes and increased body mass in the Ob mice. The effect was due to specific numbers of Bacteriodites transferred from the Ln microbiota into Ob microbiota by diet. This indicates that obesity phenotypes could be rapid, transmissible, and modifiable effects caused by interactions between diet and microbiota [7].

The diet and host digestion of carbohydrates affect the amount of end-product short-chained fatty acid (SCFA) in the stool. SCFA have shown beneficial effects on the host metabolism, normalizing glucose level, and reducing plasma levels of cholesterol [8]. Studies on mice have shown that increased production of SCFA by decreased Bacteroidetes and increased Firmicutes can promote obesity by increasing colonic energy availability [9]. A difference in gut bacterial composition between people with severe obesity and normal weight has been shown in several studies [10, 11]. The footprint shown is reduced microbial diversity and gene richness in people suffering from severe obesity. Data is 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 *Bacteroidets*, *Faecalibacterium prausnitzii* and *Akkermansia muciniphilia*. An increase in the *Firmucutes*/*Bacteroidetes* ratio is associated with obesity [10].

Hypothesis and objectives:

We hypothesize 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 by FMT can lead to weight reduction ≥ 10 %. The collection of biological materials both before and after intervention allows further analysis to elucidate the role of the gut microbiotas in obesity, energy metabolism, and immune response.

Method:

Trial design:

This is a single-center, double-blinded, placebo-controlled, parallel-group study with 12-month follow-up, performed at the medical department, 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 pre-determined in-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, 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 12months after treatment (figure 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 dialogue with the patient organization, 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 sixty participants, recruited consecutively from patients referred to our outpatient clinic for patients with severe obesity at UNN Harstad, Medical department. Patients will be asked to participate in the study if they fulfill the study criteria listed in table 1. 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 to participate in the study. Approximately one hundred new patients are referred to our outpatient obesity clinic each year.

Donors

Only individuals matching the criteria in table 1 and the European consensus criteria on fecal microbiota transplantation 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 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, UNN Harstad. We will record every reason for failure during the recruitment process.

Table 1: Inclusion and exclusion criteria for participation and fecal donors in the randomized controlled trial of fecal microbiota in severe obesity.

Study Participants	
Inclusion	**Exclusion**
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 breastfeeding
BMI > 40 kg/m2 or BMI > 35 kg/m2 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 three months or during the follow-up period that can impact on metabolism or bodyweight
	Antibiotic treatment the last three months
	Serious food allergies
Fecal Donors	
Inclusion	**Exclusion**
Age 16-40	Use of peroral antibiotics past 6 months
Sign consent form	Tattoo or piercing past 6 months
Healthy	Close relatives with serious autoimmune disease, psychiatric disorder or obesity
BMI 18 - 25	Former imprisonment
	History of Chronic diarrhea Constipation Inflammatory bowel disease Irritable bowel syndrome Colorectal polyps Immuno-suppression Obesity Metabolic syndrome CFS/ME Psychiatric disorders Other serious autoimmune disease Cancer
	High-risk sexual behavior
	Bowel movements that does not correspond to a Bristol Stool Scale type 3 or 4

	Journeys abroad the last six months to countries high in antibiotic resistance
	Use of food supplements, pre-, pro-, or symbiotics
	Dysbiosis grade 3 or more by the GA-map™ dysbiosis test [13].

Preparation of FMT transplant:

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

Donor transplant: Date of donation + DonorID

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 the University hospital of North Norway, 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 37ºC. The thawing lasts for one 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 University Hospital of North Norway 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, UNN Harstad by the research group [1]. The participants will perform a bowel lavage using Sodiumpicosulphate/Magnesiumcitrate (Picoprep, Ferring) 24 hours before delivery of FMT and take 8mg Loperamide one hour prior to the FMT procedure.

Procedure for administrating FMT

The participant will lie on his/hers left side in Trendelenburg's position while the examiner preforms a digital examination. The probe from the enema kit is lubricated and inserted in the rectum. A balloon that avoids leaking is inflated and unlocked so that the FMT can drain through the kit and to the rectum. 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 two minutes.
2. The participant turns directly to an abdominal position and holds it for 2 minutes. The head and body should still be tilted down, Trendelenburg's position.
3. The participant turns slowly until lying on the right side and holds this position for 2 minutes.
4. The bench is then tilted the opposite way (anti-Trendelenburg) and the position is held for 2 minutes.
5. The balloon around the rectal probe is deflated and removed from the rectum. The bench is tilted to a neutral position.
6. The participant is left in neutral position 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 (e.g. age, gender, height, waist circumference, and blood pressure) together with several patient reported outcome measurements (PROM`s), all listed in table 2.

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[1)]			X[1)]	X[1)]	X[1)]
15 minutes continues ECG for Heart rate variability (HRV) measurement *	X[2)]			X[2)]		

	Screening period	Treatment Period	Follow up period			
	First meeting/inclusion	Treatment day	1 month	3 months	6 months	12 months
Short difficult childhood questionnaire	X					
Binge eating questionnaire	X					X
RAND36	X					X
HSCL-25	X					X
IPAQ	X			X	X	
CTQ	X					
Questions about Covid-19 vaccination and infection[3)]	X		X	X	X	X
Questions regarding the lockdown due to Covid-19 [4)]	X		X	X	X	X
Patient-reported adverse event (AE)				X	X	X
FFQ	X			X	X	X
iDXA total body	X					X
Blood samples	X[5,6)]			X[6)]	X[6)]	X[6)]
Fecal Sample	X[7,8)]		X[8)]	X[8)]	X[8)]	X[8)]
Fecal microbiota transplantation		X				

Data capture in the eCRF Data capture on paper

1. Blood pressure, pulse, weight loss in per cent, and anthropometric measurement
2. Will be measured by the participant at home as instructed by the study personnel.
3. Included participants, and participants still in the follow up period, was asked questions about Covid-19 vaccination and covid-19 infection from mid-June – 21
4. Will only be given to participants who were in the follow-up period from march-20 to Juli-20.
5. Hb, Leucocytes, thrombocytes, folate, iron, ferritin, TIBC, vitamin B12, and vitamin D
6. hs-CRP, erythrocyte sedimentation rate (ESR), Hb, HCT, WBC, PLT, Sodium, Potassium, creatinine, ASAT, ALAT, ALP, γGT, LDL, HDL (including subgroups), triglycerides, TSH, fT4, HbA1c, C-peptide, cholesterol, amylase, fasting glucose level, insulin, quantifying the sensitivity and beta-cell function (HOMA-IR and HOMA-B) using calculators, cytokines, Tempus RNA for storage
7. SCFA, microbiota, possible placebo, and storage
8. SCFA, microbiota, and storage

*). The HRV measurement will only be performed on the last 20 participants in the study.

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 by FMT can lead to weight reduction ≥ 10 %, 12 months post FMT.

Secondary outcome:

Secondary outcomes are listed in Table 3

Table 3: Secondary endpoints, objective, and assessment.

	Objectives	Endpoints	Assessments
Secondary	Weight reduction	Changes in % body weight	We will report participants who has a weight loss of ≥ 5%, > 15% and 20%, using chi-square.
	Waist Circumference		Measured in cm at inclusion and all follow-up visits
	Gut microbiota composition and function		Identify bacterial flora using whole genome sequencing [14].
	Lipid profile will be analyzed 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 analyzed 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 (MCAF), IP-10,Eotaxin,MIP-1a,MIP-1b, RANTES, G-CSF,GM-CSF,Basic FGF, PDGF-BB, VEGF)
	Content of short chain fatty acid in feces will be analyzed in fecal samples taken 1, 3, 6 and 12 months post-FMT. fatty acid in faces		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 (ESR), 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 RAND36 questionnaire		Short form Health Survey Questionnaire (RAND 36): Quality of life will be assessed by RAND36, which is a validated instrument for general quality

			of life [15]. A score of 100 is equivalent to perfect health.
	Traumatic childhood and mental health disorder, share, correlation and relation to weight gain		Hopkins Symptoms Check List (HSCL-25): Measures general psychological distress such as anxiety and depression [16]. A score of 1,75 or above indicated physical a clinically relevant level of symptoms of depression or anxiety. Binge eating scale (BES): 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 nonintrusive items including subjective evaluations of their childhood [17]. The questionnaire have a maximal score of 20. Childhood Trauma Questionnaire (CTQ): 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 [18]. The questionnaire will be scored in categories of physical abuse (≥ 8), emotional abuse (≥ 8), sexual abuse (≥ 6), physical neglect (≥ 8), emotional neglect (≥ 10).
	Heart rate variability		Heart rate variability will be measured using Firstbeat Lifestyle Assessment at inclusion and at 3 months follow-up. The measurement will be done at home by the study participants, as instructed by study personnel. This will allow us to investigate if recovery of a 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.
	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 question (IPAQ):		Collects data on health-related physical activity [19]

Exploratory evaluation

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

Donor microbiota engraftment 1-, 3-, 6-, and 12-months post FMT:
Comparison between baseline profile, post-FMT and donor profile will show if engraftment of donor microbiota parallels clinical response to active FMT.

Questionnaire about 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.

Heart rate variability:
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 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, standard deviation near 7. This will therefore be the expected result in the control group (receiving placebo). A weight reduction ≥ 10% leads to significant improvement of health and quality of life [20], and a weight change of this magnitude is therefore considered clinically relevant. The difference between the two groups is estimated to 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, significance level 0.05. We will eliminate extreme values; more than 3 SD out of the average in the group. In this patient group, we must also be prepared to high degree loss of follow-up near one third, and as this is our experience from regular follow of patients at the out patient clinic for severe obesity [21]. Therefore, we will include 60 participants, 30 in each group.

Randomization and blinding

A research nurse at the Department of Clinical Research at the University Hospital of North Norway, Harstad (UNN Harstad) creates the allocation sequence using the REDCap software. The treatment is randomized 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 randomize participants

The allocator uses the randomization 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 randomized to active treatment will have their tag on the placebo transplants switched to the donor transplant by the allocator upon allocation. The placebo transplant will be disposed 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 studyID 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 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

Participants, investigators, and outcome assessors are kept blind to the allocation and intervention. One person will have the designated task of allocating treatment to participants and is kept blind by not knowing the corresponding participant's identity to the studyID. The only personnel that will have access to the randomization sequence at the REDCap software is the allocator (UNN, Harstad). The allocator will not have any access to the participants, be involved in inclusion, assigning of treatment, follow up, or data handling at the end of the trial. If any adverse events, the Principal investigator (UNN, Harstad) has the authority to emergency unblind. This will be followed by an adverse reaction report. The Principal investigator (UNN Harstad) will decide if it is necessary to unblind participants or the study personnel involved in inclusion, randomization, allocation, assigning of treatment, or follow-up.

Statistical method and data analysis

Primary analysis

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 ≥ 10% in the intervention group, will be presented as bar charts with comparison between the intervention and control group. Chi Square or Fisher's exact 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 end point including only the participants who completed the study.

We will do a two-side t-test (Students t-test) comparing weight loss percent 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 analyze metagenomic data in the pipeline described in earlier publication [14]

Repeated measures ANOVA will be used to measure the effect of FMT on RAND36. 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. 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 after 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 HbA1c before FMT and 3, 6, and 12 months after FMT. The sensitivity and beta-cell function (HOMA-IR and HOMA-B) 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, to lifestyle changes and eating habits.

Short difficult childhood questionnaire, CTQ, and HSCL-25 will be analyzed as numbers with a positive score, share, and contribution, and correlation between them. Further, we will analyze to which degrees these parameters affect weight reduction at the end of follow-up.

The statistical analyzes 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:

Voluntary discontinuation by the participant who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
Safety reason as judged by the Principal Investigator
Major protocol deviation
Incorrect enrollment i.e., the participant does not meet the required inclusion/exclusion criteria for the study
Participant lost to follow-up
Pregnancy
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:
Occurrence of adverse event (AE) unknown to date in respect of their nature, severity, and duration
Medical or ethical reasons affecting the continued performance of the trial
Difficulties in the recruitment of participants
The sponsor and principal investigator(s) 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 adverse events using enema in fecal microbiota transplantation. The procedure is non-invasive, uses minimal recourses and is found to be safe. The study was approved 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 adverse events (AE) are reported, PCV and HMH evaluate and involve RG and BK if necessary. If any serious adverse events (SAE) FMT will be stopped until the board has discussed further measures. Patient-reported adverse events will be documented in a separate questionnaire. A suspect adverse reaction report will follow any suspicion of an adverse event. In addition to asking for patient-reported adverse events 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. Serious adverse events or symptom deterioration for participants will prompt evaluation for opening the randomization sequence and premature termination of the study are reported, 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 studyID is used for all documentation, reports and publication. Data is collected using REDCap software. Patient reported outcomes are obtained in the e-CRF, except for the FFQ. All data is stored for 15 years, and the biological material is stored for five years on 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 day 365 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 are in the follow up process. The last participants last visit will be in August 2023. The reason that the study protocol has not been sent for publication earlier is due to difficulties in the 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.

Author contributions:

PCV, MSF, PHJ, BK, and RG have contributed to secure research funding.

HMH, PCV, MSF, LCKS, PHJ, KA and BK have been part of the finalization of the study design and writing of the protocol.

LCKS, PHJ and HMH planned and performed the donor screening program and were responsible for the implementation of the program.

HMH is the PhD student in the project and has had the final reviewing of the protocol.

All authors critically revised the protocol, gave their final approval, and agree to be accountable for all aspects and ensure integrity and accuracy.

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Declaration of conflicting interests:

The authors have declared these conflict of interests:

Peter Holger Johnsen: Principal Investigator in REFIT 2 trial. An investigator-initiated and-run randomized controlled trial investigating fecal microbiota transplantation by enema in patients with rirritable bowel syndrome.

Maria Serafia Fjellstad: Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Novo Nordisk.

Hege Marie Hanssen: Subinvestigator in “fecal microbiota transplantation in CFS/ME”, Member scientific committee in REFIT2 trial, Member scientific committee in COLONIZE trial

Patient consent:

All study participants receive information and sign a consent form before they are included in the study.

Ethics approval:

The study has approval from the Medical and Health Research Ethics (2017/1655/REK nord) and privacy representative at the University Hospital of North Norway.

Provenance and peer review:
Not commissioned; externally peer reviewed.

Reference:

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622

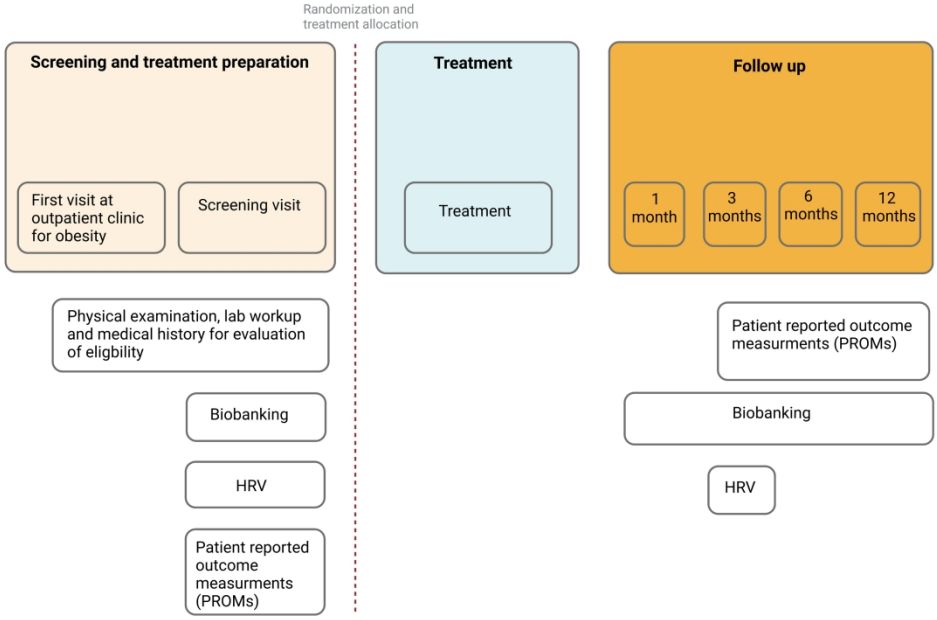

Figure 1: Patient flow and data capture, redrawn from C.O.L.O.N.I.Z.E/ Fredrik Juul, created with BioRender.com

266x170mm (300 x 300 DPI)

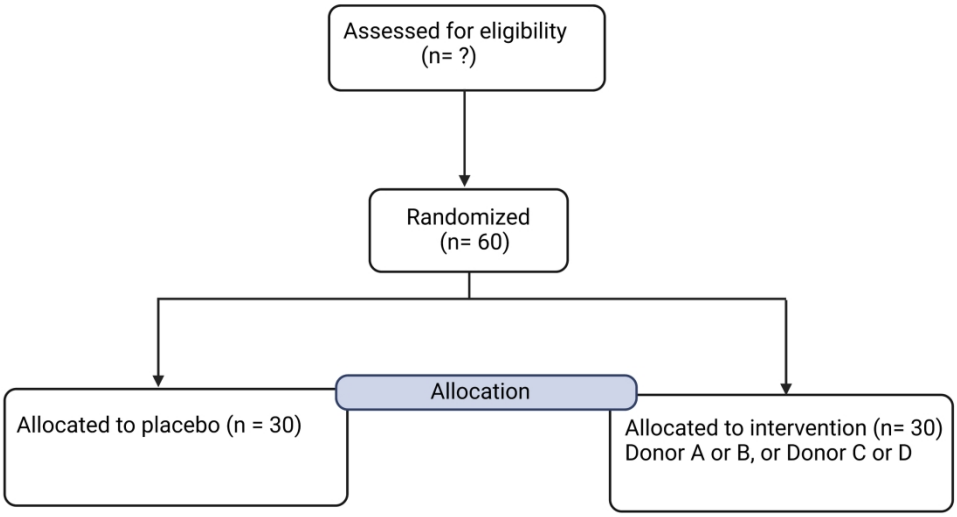

Figure 2: Study flow chart, created with BioRender.com

224x126mm (300 x 300 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6
	2b	Specific objectives or hypotheses	6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6, 7 and 8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	8 and 9
	4b	Settings and locations where the data were collected	8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	10 and 11
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	12, 13, 14 and 15
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	16
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	16 and 17
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	16
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	16
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8, 16 and 17
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	16 and 17

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	17 and 18
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	17 and 18
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	3

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Randomized, placebo-controlled, double-blinded trial of fecal microbiota transplantation in severe obesity: A Study protocol

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Title page

Randomized, 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 co-morbidities. Options are conservative treatment such as life style changes, bariatric surgery or medications. Conservative treatment has a low success rate, and bariatric surgery is irreversible, 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 do 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 life style intervention, contribute to weight reduction in people suffering from severe obesity.

Method and analysis:

This study aims to investigate if a transfer of fecal microbiota (FMT) from a lean donor leads to weight reduction in participants suffering from severe obesity. The study is a single-center, double-blinded, placebo-controlled, parallel-group study with 60 participants. Participants will be randomized 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, Medical department, University Hospital North Norway, by invitation only. The study has a follow-up period of 12 months, with study visits 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:

The trial is registered at clinicaltrials.gov with trial registration number NCT 03273855.

Strenghts and limitations:

- 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 randomized 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 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 allows 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 the both the functional potential and bacterial composition of the microbiome in severe obesity.

Keywords

Severe obesity, fecal microbiota transplantation, weight reduction, gut microbiota, lifestyle intervention.

Introduction

The gut microbiota is recognized 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* (CDI) [2]. Recent studies have shown that alterations in the composition of the gut microbiota could be the cause of several diseases [3, 4]. Pathological conditions in the gastrointestinal, nervous, and respiratory systems could occur if the equilibrium between the host and microbiota is disturbed [5].

A paper from 2014 by Ridaura. et.al showed that obesity-associated metabolic phenotypes, total body, and fat mass was transmissible from twins discordant for obesity to germ free mice. Co-housing mice who had received microbiota from the obese twin (Ob) with mice who had received the lean twin's microbiota (Ln) prevented development of obesity associated phenotypes and increased body mass in the Ob mice. The effect was due to specific numbers of Bacteriodites transferred from the Ln microbiota into Ob microbiota by diet. This indicates that obesity phenotypes could be rapid, transmissible, and modifiable effects caused by interactions between diet and microbiota [6].

The diet and host digestion of carbohydrates affect the amount of end-product short-chained fatty acid (SCFA) in the stool. SCFA have shown beneficial effects on the host metabolism, normalizing 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 is 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 *Bacteroidets*, *Faecalibacterium prausnitzii* and *Akkermansia muciniphilia*. An increase in the *Firmucutes*/*Bacteroidetes* ratio is associated with obesity [9].

People suffering from severe obesity has a systemic low-grade inflammation. Maachi. Et. al showed in a study of 15 obese women (BMI > 32), a strong relationship between inflammatory markers and adipocytokines. The study showed that systemic low- grade inflammation was related to both circulating and adipose tissue TNFα, IL-6 and leptin, suggesting that secretion of cytokines by adipose tissue could play a role in increased inflammatory preteins secreted by the liver in people suffering from obesity [11].

Our study will use hs-CRP (high sensitive C-reactive protein) as a screening test for this low-grade inflammation. A complete cytokine analysis will give further dept 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 hypothesize 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 by FMT can lead to weight reduction ≥ 10 %. The collection of biological materials both before and after intervention allows further analysis to elucidate the role of the gut microbiotas in obesity, energy metabolism, and immune response.

Method:

Trial design:

This is a single-center, double-blinded, placebo-controlled, parallel-group study with 12-month follow-up, performed at the medical department, 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 pre-determined in-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, 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 12months after treatment (figure 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 dialogue with the patient organization, 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 sixty participants, recruited consecutively from patients referred to our outpatient clinic for patients with severe obesity at UNN Harstad, Medical department. At the outpatient clinic, all the participants will undergo a 12-month lifestyle change program while participating in the study. Patients will be asked to participate in the study if they fulfill the study criteria listed in table 1. 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 to participate in the study. Approximately one hundred new patients are referred to our outpatient obesity clinic each year.

Donors

Only individuals matching the criteria in table 1 and the European consensus criteria on fecal microbiota transplantation 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, UNN Harstad. We will record every reason for failure during the recruitment process.

Table 1: Inclusion and exclusion criteria for participation and fecal donors in the randomized controlled trial of fecal microbiota in severe obesity.

Study Participants	
Inclusion	**Exclusion**
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 breastfeeding
BMI > 40 kg/m2 or BMI > 35 kg/m2 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 three months or during the follow-up period that can impact on metabolism or bodyweight
	Antibiotic treatment the last three months
	Serious food allergies
Fecal Donors	
Inclusion	**Exclusion**

Age 16-40	Use of peroral antibiotics past 6 months
Sign consent form	Tattoo or piercing past 6 months
Healthy	Close relatives with serious autoimmune disease, psychiatric disorder or obesity
BMI 18 - 25	Former imprisonment
	History of Chronic diarrhea Constipation Inflammatory bowel disease Irritable bowel syndrome Colorectal polyps Immuno-suppression Obesity Metabolic syndrome CFS/ME Psychiatric disorders Other serious autoimmune disease Cancer
	High-risk sexual behavior
	Bowel movements that does not correspond to a Bristol Stool Scale type 3 or 4
	Journeys abroad the last six months to countries high in antibiotic resistance
	Use of food supplements, pre-, pro-, or symbiotics
	Positive cultures for ß-lactamase producing Enterobacteriaceae (ESBL-E), vancomycin-resistant Enterococci (VRE) and methicillin-resistant S. Aureus (MRSA), Salmonella spp, Shigella spp, Campylobacter spp,Yersinia spp, and toxin-producing C difficile. Fecal tests for, viruses (norovirus, rotavirus, Sapovirus, adenovirus), and occult blood and E. GA-map™ dysbiosis test:with dysbiosis grade 3 or more by the GA-mapTM dysbiosis test [1)] [13].
	Blood samples for glycated haemoglobin, serology for HIV, Treponema pallidum and hepatitis A, B, C, and E.

1) 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.

Preparation of FMT transplant:

Fresh donated feces (50 gram) are mixed with 25mL 85% glycerol and 120mL saline to a homogenized solution, and poured through a 0.5 mm mesh strainer. The solution is transferred to four 50 mL Luerlock 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

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 the University hospital of North Norway, 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 one 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 University Hospital of North Norway 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, UNN Harstad by the research group [14]. The participants will perform a bowel lavage using Sodiumpicosulphate/Magnesiumcitrate (Picoprep, Ferring) 24 hours before delivery of FMT and take 8mg Loperamide one hour prior to the FMT procedure.

Procedure for administrating FMT

The participant will lie on his/hers left side in Trendelenburg's position while the examiner preforms a digital examination. The probe from the enema kit is lubricated and inserted in the rectum. A balloon that avoids leaking is inflated and unlocked so that the FMT can drain through the kit and to the rectum. 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 two minutes.
2. The participant turns directly to an abdominal position and holds it for 2 minutes. The head and body should still be tilted down, Trendelenburg's position.
3. The participant turns slowly until lying on the right side and holds this position for 2 minutes.
4. The bench is then tilted the opposite way (anti-Trendelenburg) and the position is held for 2 minutes.

5. The balloon around the rectal probe is deflated and removed from the rectum. The bench is tilted to a neutral position.
6. The participant is left in neutral position 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 (e.g. age, gender, height, waist circumference, and blood pressure) together with several patient reported outcome measurements (PROM`s), all listed in table 2.

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[1)]			X[1)]	X[1)]	X[1)]
15 minutes continues ECG for Heart rate variability (HRV) measurement *	X[2)]			X[2)]		
Short difficult childhood questionnaire	X					
Binge eating questionnaire	X					X
RAND36	X					X
HSCL-25	X					X
IPAQ	X			X	X	
CTQ	X					
Questions about Covid-19 vaccination and infection[3)]	X		X	X	X	X
Questions regarding the lockdown due to Covid-19 [4)]	X		X	X	X	X
Patient-reported adverse event (AE)				X	X	X

	Screening period	Treatment Period	Follow up period			
	First meeting/inclusion	Treatment day	1 month	3 months	6 months	12 months
FFQ	X			X	X	X
iDXA total body	X					X
Blood samples	X[5,6)]			X[6)]	X[6)]	X[6)]
Fecal Sample	X[7,8)]		X[8)]	X[8)]	X[8)]	X[8)]
Fecal microbiota transplantation		X				

Data capture in the eCRF Data capture on paper

1. Blood pressure, pulse, weight loss in per cent, and anthropometric measurement
2. Will be measured by the participant at home as instructed by the study personnel.
3. Included participants, and participants still in the follow up period, was asked questions about Covid-19 vaccination and covid-19 infection from mid-June – 21
4. Will only be given to participants who were in the follow-up period from march-20 to Juli-20.
5. Hb, Leucocytes, thrombocytes, folate, iron, ferritin, TIBC, vitamin B12, and vitamin D
6. hs-CRP, erythrocyte sedimentation rate (ESR), Hb, HCT, WBC, PLT, Sodium, Potassium, creatinine, ASAT, ALAT, ALP, γGT, LDL, HDL (including subgroups), triglycerides, TSH, fT4, HbA1c, C-peptide, cholesterol, amylase, fasting glucose level, insulin, quantifying the sensitivity and beta-cell function (HOMA-IR and HOMA-B) using calculators, cytokines, Tempus RNA for storage
7. SCFA, microbiota, possible placebo, and storage
8. SCFA, microbiota, and storage

*). The HRV measurement will only be performed on the last 20 participants in the study.

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 by FMT can lead to weight reduction ≥ 10 %, 12 months post FMT.

Secondary outcome:

Secondary outcomes are listed in Table 3

Table 3: Secondary endpoints, objective, and assessment.

	Objectives	**Endpoints**	**Assessments**
Secondary	Weight reduction	Changes in % body weight	We will report participants who has a weight loss of ≥ 5%, > 15% and 20%, using chi-square.
	Waist Circumference		Measured in cm at inclusion and all follow-up visits
	Gut microbiota composition and function		Identify bacterial flora using metagenome sequencing [15].
	Lipid profile will be analyzed in blood samples taken 3, 6 and		Cholesterol, LDL, HDL including subgroups, triglycerides (Collaboration with Nordlands

	12 months post-FMT		hospital in Bodø
	A Cytokine panel will be analyzed 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 (MCAF), IP-10,Eotaxin,MIP-1a,MIP-1b, RANTES, G-CSF,GM-CSF,Basic FGF, PDGF-BB, VEGF)
	Content of short chain fatty acid in feces will be analyzed in fecal samples taken 1, 3, 6 and 12 months post-FMT. fatty acid in faces		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 (ESR), 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 RAND36 questionnaire		Short form Health Survey Questionnaire (RAND 36): Quality of life will be assessed by RAND36, which is a validated instrument for general quality of life [16]. A score of 100 is equivalent to perfect health.
	Traumatic childhood and mental health disorder, share, correlation and relation to weight gain		Hopkins Symptoms Check List (HSCL-25): Measures general psychological distress such as anxiety and depression [17]. A score of 1,75 or above indicated physical a clinically relevant level of symptoms of depression or anxiety. Binge eating scale (BES): 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 nonintrusive items including subjective evaluations of their childhood [18]. The questionnaire have a maximal

			score of 20. Childhood Trauma Questionnaire (CTQ): 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 [19]. The questionnaire will be scored in categories of physical abuse (≥ 8), emotional abuse (≥ 8), sexual abuse (≥ 6), physical neglect (≥ 8), emotional neglect (≥ 10).
	Heart rate variability		Heart rate variability will be measured using Firstbeat Lifestyle Assessment at inclusion and at 3 months follow-up. The measurement will be done at home by the study participants, as instructed by study personnel. This will allow us to investigate if recovery of a 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.
	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 question (IPAQ):		Collects data on health-related physical activity [20]

Exploratory evaluation

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

Donor microbiota engraftment 1-, 3-, 6-, and 12-months post FMT:
Comparison between baseline profile, post-FMT and donor profile will show if engraftment of donor microbiota parallels clinical response to active FMT.

Questionnaire about 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.

Heart rate variability:
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 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, standard deviation near 7. This will therefore be the expected result in the control group (receiving placebo). A weight reduction ≥ 10% leads to significant improvement of health and quality of life [21], and a weight change of this magnitude is therefore considered clinically relevant. The difference between the two groups is estimated to 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, significance level 0.05. We will eliminate extreme values; more than 3 SD out of the average in the group. In this patient group, we must also be prepared to high degree loss of follow-up near one third, and as this is our experience from regular follow of patients at the out patient clinic for severe obesity [22]. Therefore, we will include 60 participants, 30 in each group.

Randomization and blinding

A research nurse at the Department of Clinical Research at the University Hospital of North Norway, Harstad (UNN Harstad) creates the allocation sequence using the REDCap software. The treatment is randomized 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 randomize participants

The allocator uses the randomization 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 randomized to active treatment will have their tag on the placebo transplants switched to the donor transplant by the allocator upon allocation. The placebo transplant will be disposed 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 studyID 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 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

Participants, investigators, and outcome assessors are kept blind to the allocation and intervention. One person will have the designated task of allocating treatment to participants and is kept blind by not knowing the corresponding participant's identity to the studyID. The only personnel that will have access to the randomization sequence at the REDCap software is the allocator (UNN, Harstad). The allocator will not have any access to the participants, be involved in inclusion, assigning of treatment, follow up, or data handling at the end of the trial. If any adverse events, the Principal investigator (UNN, Harstad) has the authority to

emergency unblind. This will be followed by an adverse reaction report. The Principal investigator (UNN Harstad) will decide if it is necessary to unblind participants or the study personnel involved in inclusion, randomization, allocation, assigning of treatment, or follow-up.

Statistical method and data analysis

Primary analysis

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 ≥ 10% in the intervention group, will be presented as bar charts with comparison between the intervention and control group. Chi Square or Fisher's exact 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 end point including only the participants who completed the study.

We will do a two-side t-test (Students t-test) comparing weight loss percent 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 analyze metagenomic data in the pipeline described in earlier publication [15]

Repeated measures ANOVA will be used to measure the effect of FMT on RAND36. 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. 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 after 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 HbA1c before FMT and 3, 6, and 12 months after FMT. The sensitivity and beta-cell function (HOMA-IR and HOMA-B) 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, to lifestyle changes and eating habits.

Short difficult childhood questionnaire, CTQ, and HSCL-25 will be analyzed as numbers with a positive score, share, and contribution, and correlation between them. Further, we will analyze to which degrees these parameters affect weight reduction at the end of follow-up.

The statistical analyzes 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:

Voluntary discontinuation by the participant who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
Safety reason as judged by the Principal Investigator
Major protocol deviation
Incorrect enrollment i.e., the participant does not meet the required inclusion/exclusion criteria for the study
Participant lost to follow-up
Pregnancy
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:
Occurrence of adverse event (AE) unknown to date in respect of their nature, severity, and duration
Medical or ethical reasons affecting the continued performance of the trial
Difficulties in the recruitment of participants
The sponsor and principal investigator(s) 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 adverse events using enema in fecal microbiota transplantation. The procedure is non-invasive, uses minimal recourses and is found to be safe. The study was approved 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 adverse events (AE) are reported, PCV and HMH evaluate and involve RG and BK if necessary. If any serious adverse events (SAE) FMT will be stopped until the board has discussed further measures. Patient-reported adverse events will be documented in a separate questionnaire. A suspect adverse reaction report will follow any suspicion of an adverse event. In addition to asking for patient-reported adverse events 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. Serious adverse events or symptom deterioration for participants will prompt evaluation for opening the randomization sequence and premature termination of the study are reported, 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 studyID is used for all documentation, reports and publication. Data is collected using REDCap software. Patient reported outcomes are obtained in the e-CRF, except for the FFQ. All data is stored for 15 years, and the biological material is stored for five years on 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 day 365 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 are in the follow up process. The last participants last visit will be in August 2023. The reason that the study protocol has not been sent for publication earlier is due to difficulties in the 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.

Author contributions:

PCV, MSF, PHJ, BK, and RG have contributed to secure research funding.

HMH, PCV, MSF, LCKS, PHJ, KA and BK have been part of the finalization of the study design and writing of the protocol.

LCKS, PHJ and HMH planned and performed the donor screening program and were responsible for the implementation of the program.

HMH is the PhD student in the project and has had the final reviewing of the protocol.

All authors critically revised the protocol, gave their final approval, and agree to be accountable for all aspects and ensure integrity and accuracy.

Funding:

This work was supported by Northern Norway Regional Health Authority (Grant number HNF1411-18).

Declaration of conflicting interests:

The authors have declared these conflict of interests:

Peter Holger Johnsen: Principal Investigator in REFIT 2 trial. An investigator-initiated and-run randomized controlled trial investigating fecal microbiota transplantation by enema in patients with rirritable bowel syndrome.

Maria Serafia Fjellstad: Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Novo Nordisk.

Hege Marie Hanssen: Subinvestigator in “fecal microbiota transplantation in CFS/ME”, Member scientific committee in REFIT2 trial, Member scientific committee in COLONIZE trial

Patient consent:

All study participants receive information and sign a consent form before they are included in the study.

Ethics approval:

The study has approval from the Medical and Health Research Ethics (2017/1655/REK nord) and privacy representative at the University Hospital of North Norway.

Provenance and peer review:
Not commissioned; externally peer reviewed.

Reference:

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Figure legend:

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

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

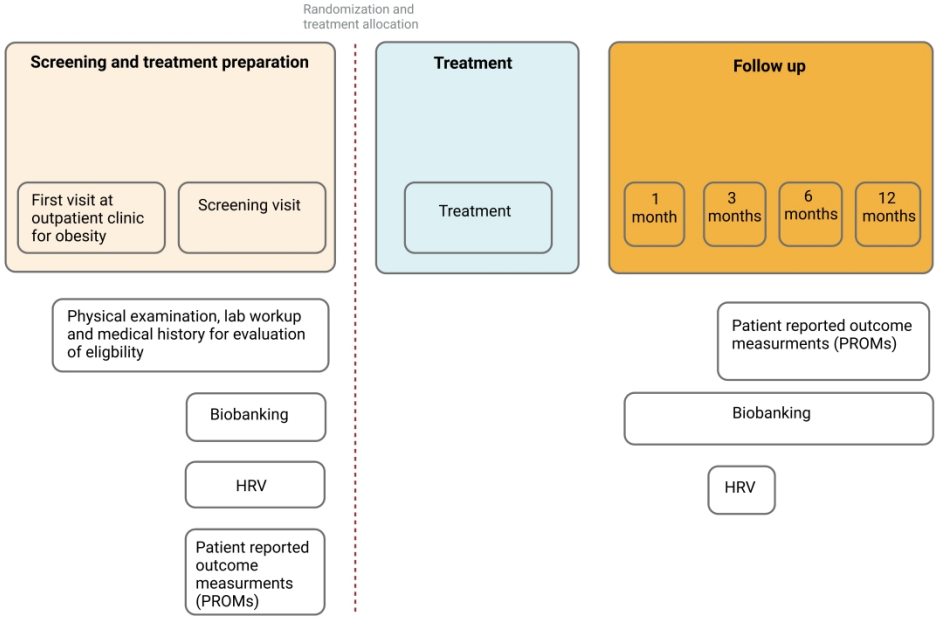

Figure 1: Patient flow and data capture, redrawn from C.O.L.O.N.I.Z.E/ Fredrik Juul, created with BioRender.com

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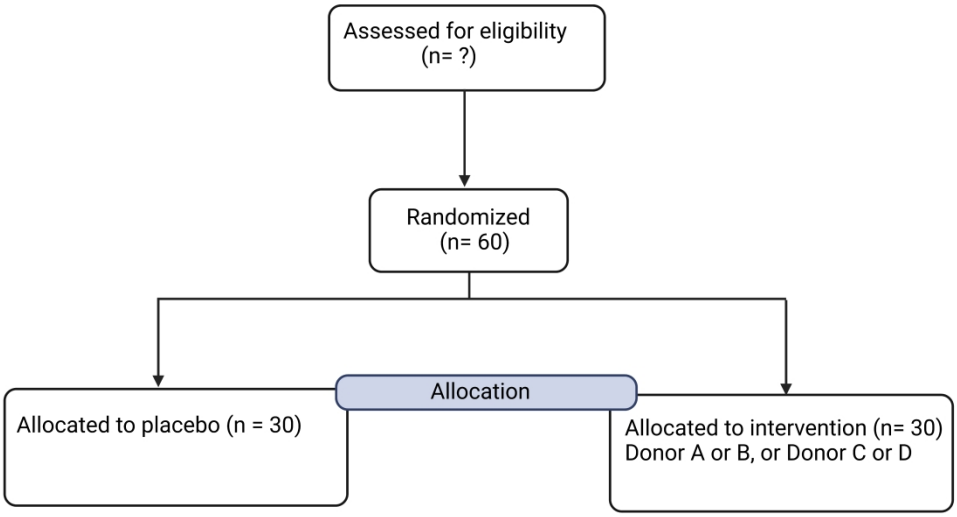

Figure 2: Study flow chart, created with BioRender.com

224x126mm (600 x 600 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Page: 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Page: 5
	2b	All items from the World Health Organization Trial Registration Data Set N/A
Protocol version	3	Date and version identifier Page: 3
Funding	4	Sources and types of financial, material, and other support Page: 3
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Page 1 and 2.
	5b	Name and contact information for the trial sponsor Page 3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Page: 19

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
		N/A
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
		Page: 6
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
		Page: 6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
		Page: 7, 8, 9, 10, 14 and 15.
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
		Page: 7
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)
		Page: 8 and 9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
		Page: 10
	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)
		Page: 16 and 17.

	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial Page: 8
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 Page: 12
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) Page: 11
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 Page: 14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size:

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Page: 15

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Page: 15
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Page: 15
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Page: 15
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Page: 16 and 17.
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Page: 10 and 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols N/A
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol ???

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Page: 15 and 16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) N/A
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) N/A
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct N/A
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor N/A
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Page: 19

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) ???
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Page: 7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable N/A
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 Page: 11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Page: 19
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators N/A
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 N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Page: 18
	31b	Authorship eligibility guidelines and any intended use of professional writers N/A

	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code N/A
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates Written in Norwegian, can be translated and given upon request.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Page: 9

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.