PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Randomized, placebo-controlled, double-blinded trial of fecal microbiota transplantation in severe obesity: A Study protocol
AUTHORS	Hanssen, Hege Marie; Fjellstad, Maria; Skjevling, Linn; Johnsen, Peter Holger; Kulseng, Bård; Goll, Rasmus; Almå, Kristin; Valle, Per-Christian

VERSION 1 – REVIEW

REVIEWER	Perttu Lahtinen Päijät-Häme Central Hospital
REVIEW RETURNED	28-May-2023

GENERAL COMMENTS	Dear Authors, The interaction of gut microbiota and metabolism and body weight is very important subject to study. The study protocol is very well designed. The outcome measures are comprehensive. The intervention, the way of administering FMT, is very innovative and it is important to study in which extent it is able to result engraftment of donor microbiota into the patient. It would be interesting to hear previous experiences of the used FMT protocol. In previous controlled trials on FMT for obesity (Lahtinen et al. JAMA Netw Open. 2022 Dec 1;5(12):e2247226 and Leong et al. JAMA Netw Open. 2020 Dec 1;3(12):e2030415.) , FMT did not have a significant effect on weight in human subjects. Thus, I find it unlikely that the primary endpoint of 10% re-duction in the %TWL will be reached with a single FMT. However, in diseases other then Clostridioides difficile there may be a substantial difference between donors (Moayyedi et al. Gastroenterology. 2015 Jul;149(1):102-109.). And the protocol of administering FMT is different in this study compared to previous RCT's. Therefore, I think the primary endpoint is relevant. The authors could consider stating, how many donors will be used in this study. Are the study patients receiving dietary or other therapy during the follow up? This should be stated and described. In the Abstract it is stated: "Obesity is the main threat… " Obesity is a severe threat, but to state it is the main threat, may underestimate some other severe threats. This is a well-designed study that will provide important new knowledge of the interaction between gut microbiota and

	metabolism.

REVIEWER	Bogdan Neamtu Lucian Blaga University of Sibiu, Clinical Department Faculty of Medicine, Pediatric Clinical Hospital from Sibiu, Romania
REVIEW RETURNED	06-Jun-2023

GENERAL COMMENTS	Dr. Hege Marie Hanssen and her colleagues propose a proof-of-concept study that aims to investigate the potential weight reduction in individuals with severe obesity following fecal microbiota transplant (FMT) from lean donors. The study design entails a single-center, double-blinded, placebo-controlled, parallel-group study with 60 donors. Participants will be randomized in a 1:1 ratio to receive either FMT (enema) from a lean donor or placebo. The follow-up period will span 12 months, with study visits scheduled at 3, 6, and 12 months post-FMT. The primary endpoint of the study is a reduction of at least 10% in body weight by the end of the intervention. This study design holds promise, as previous research on obesity and gut microbiota has shown encouraging results in terms of weight reduction and improvements in metabolic health. However, before considering its publication, there are several issues that need to be addressed appropriately: 1. Introduction: • The authors should provide a concise paragraph that provides an update on the interplay of cytokines and hs-PCR. This addition will help readers understand why the specific cytokine panel, along with inflammatory markers mentioned on page 12 as the secondary outcome (lines 29-36), were chosen. 2. Materials and Methods: 2.1 Exclusion criteria for donors: • In Table 3, in addition to dysbiosis grade 3, the authors should mention or at least further point out to the following criteria found later on in the Amendments section: 1 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; 2. fecal tests for, viruses (norovirus, rotavirus, Sapovirus, adenovirus,), and occult blood; 3. blood samples for glycated haemoglobin; 4. serology for HIV, Treponema pallidum and hepatitis A, B, C, and E. GA-map™ dysbiosis test: • In reference to the GA-map™ dysbiosis test, it should be noted that the bacteria measured with this method do not represent the entire phyla but only specific parts of the phyla. This limitation regarding the inclusion criteria should be clearly stated. 2.2 Power analysis (variability): • Although the standard deviation of approximately 7% is mentioned, it is unclear whether this value is based on previous studies, pilot data, or an estimate. Providing more information on how this standard deviation was determined or validated would enhance the study design. 2.3 The allocation sequence using REDCap software: • The authors have designed an allocation sequence that randomizes the treatment in fixed blocks of four, with two active donors (donor A and donor B or donor C and donor D) and two placebos. This method ensures a balanced distribution of participants and supports the validity and reliability of the results. 2.4 Statistical tests: • It is unclear whether the authors plan to use ANOVA as a

	multivariate repeated measures test or a multivariate linear mixed-effects model to analyze the data, particularly for the variables from the cytokine panel, fecal short-chain fatty acid (SCFA), insulin resistance, blood pressure, inflammation markers, and biochemical parameters of hepatic steatosis will be assessed at inclusion, 3, 6, and 12 months. 2.5 How does this study design tackle the management of cases that might experience weight gain after the FMT procedure? Addressing these concerns will enhance the clarity, and scientific rigor, of the study design.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Perttu Lahtinen, Päijät-Häme Central Hospital
Comments to the Author:
Dear Authors,

The interaction of gut microbiota and metabolism and body weight is very important subject to study. The study protocol is very well designed. The outcome measures are comprehensive.

1. The intervention, the way of administering FMT, is very innovative and it is important to study in which extent it is able to result engraftment of donor microbiota into the patient. It would be interesting to hear previous experiences of the used FMT protocol.

At our department, we have done a study investigating the way of administrating FMT, delivery by colonoscopy compared to administration by enema with and without positioning of the patient. The study showed that delivery by gastroscopy was superior to eneme in reaching the cecum of the colon. But enema with positioning was superior to enema without positioning in reaching cecum of the colon, and can be a good alternative to colonoscopy for delivery of FMT. The article is currently in revive at BMC gastroenterology (ref: 371106c8-cae6-468f-afca-b935a2298be7)

The follow up period of 12-months and checkpoint at 1, 3, 6 and 12-months post FMT will allow us to follow the microbiome of the participants until 12-months after the intervention. This gives us the opportunity to see how long the donor microbiota is engrafted in the participants receiving donor FMT, and if the engraftment correlated with the weight loss.

2. In previous controlled trials on FMT for obesity (Lahtinen et al. JAMA Netw Open. 2022 Dec 1;5(12):e2247226 and Leong et al. JAMA Netw Open. 2020 Dec 1;3(12):e2030415.) , FMT did not have a significant effect on weight in human subjects. Thus, I find it unlikely that the primary endpoint of 10% re-duction in the %TWL will be reached with a single FMT. However, in diseases other then Clostridioides difficile there may be a substantial difference between donors (Moayyedi et al. Gastroenterology. 2015 Jul;149(1):102-109.). And the protocol of administering FMT is different in this study compared to previous RCT's. Therefore, I think the primary endpoint is relevant.

We would like to thank the reviewer for the confident in the primary endpoint. As pointed out, our study protocol differs from previously published RCT on FMT and obesity. For example, the study performed by Lahtinen et.al, uses oral administration of FMT and only has a 6-month follow up period. The study performed by Leong et. al, used oral capsules for FMT and has a follow up period of 26 weeks. It is therefore difficult to compare the endpoint of those studies, to ours. The FMT solution used by Lahtinen et.al, consists of 30g feces mixed with glycerol and saline. In the study by Leong et.al, the participants received 28 capsules with a total feces content of 22 grams.

We are using a rectal administration of FMT, and our follow up period is 12-months which could greatly affect the endpoint of 10% weight loss in the intervention group. Our FMT solution consists of 50g feces mixed with glycerol and saline, which in theory could make the solution more potent.

3. The authors could consider stating, how many donors will be used in this study.

Stated under the headline “donors” at page 8- Line added: “The study plans to use 2 donors, treating 15 participants with donor A and 15 participants with donor B.”

4. Are the study patients receiving dietary or other therapy during the follow up? This should be stated and described.

Stated in the headline “study participants” at page 7. Line added: “At the outpatient clinic, all the participants will undergo a 12-month lifestyle change program while participating in the study.”

5. In the Abstract it is stated: ”Obesity is the main threat… “ Obesity is a severe threat, but to state it is the main threat, may underestimate some other severe threats.

Under the heading “abstract/introduction” at page 4, the words “one of” is added before main threat.

Reviewer: 2
Dr. Bogdan Neamtu, Lucian Blaga University of Sibiu
Comments to the Author:
Dr. Hege Marie Hanssen and her colleagues propose a proof-of-concept study that aims to investigate the potential weight reduction in individuals with severe obesity following fecal microbiota transplant (FMT) from lean donors. The study design entails a single-center, double-blinded, placebo-controlled, parallel-group study with 60 donors. Participants will be randomized in a 1:1 ratio to receive either FMT (enema) from a lean donor or placebo. The follow-up period will span 12 months, with study visits scheduled at 3, 6, and 12 months post-FMT. The primary endpoint of the study is a reduction of at least 10% in body weight by the end of the intervention.
This study design holds promise, as previous research on obesity and gut microbiota has shown encouraging results in terms of weight reduction and improvements in metabolic health. However, before considering its publication, there are several issues that need to be addressed appropriately:

1. Introduction:
The authors should provide a concise paragraph that provides an update on the interplay of cytokines and hs-PCR. This addition will help readers understand why the specific cytokine panel, along with inflammatory markers mentioned on page 12 as the secondary outcome (lines 29-36), were chosen.

On page 6, these two paragraphs were added:
“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 proteins secreted by the liver in people suffering from obesity.”
“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. “

2. Materials and Methods:

2.1 Exclusion criteria for donors:

• In Table 3, in addition to dysbiosis grade 3, the authors should mention or at least further point out to the following criteria found later on in the Amendments section: 1 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; 2. fecal tests for, viruses (norovirus, rotavirus, Sapovirus, adenovirus,), and occult blood; 3. blood samples for glycated haemoglobin; 4. serology for HIV, Treponema pallidum and hepatitis A, B, C, and E. GA-map™ dysbiosis test:

In table 1 there is added one column with “blood samples for glycated haemoglobin, serology for HIV, Treponema pallidum and hepatitis A, B, C, and E.” and in the colums with the dysbiose test, all the other fecal test are added. Page 9.

3. In reference to the GA-map™ dysbiosis test, it should be noted that the bacteria measured with this method do not represent the entire phyla but only specific parts of the phyla. This limitation regarding the inclusion criteria should be clearly stated.

Under table 1, the line “The bacteria measured with the GA-mapTM 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” is added. Page 9.

4. 2.2 Power analysis (variability):

• Although the standard deviation of approximately 7% is mentioned, it is unclear whether this value is based on previous studies, pilot data, or an estimate. Providing more information on how this standard deviation was determined or validated would enhance the study design.

The standard deviation of approximately 7% is based on 87 patients completing a 12-month lifestyle change program at the medical department, UNN Harstad. This is the same life-style change program that the study participants participate in while in the study.

5. 2.3 The allocation sequence using REDCap software:

• The authors have designed an allocation sequence that randomizes the treatment in fixed blocks of four, with two active donors (donor A and donor B or donor C and donor D) and two placebos. This method ensures a balanced distribution of participants and supports the validity and reliability of the results.

The study group is thankful for the appraisal.

6. 2.4 Statistical tests:

• It is unclear whether the authors plan to use ANOVA as a multivariate repeated measures test or a multivariate linear mixed-effects model to analyze the data, particularly for the variables from the cytokine panel, fecal short-chain fatty acid (SCFA), insulin resistance, blood pressure, inflammation markers, and biochemical parameters of hepatic steatosis will be assessed at inclusion, 3, 6, and 12 months.

The results in the study will generate an enormous amount of data, and all of the statistical methods are not pre-determined. We will employ a biostatistician to help with the interpretation of the metagenome sequencing analysis.

7. 2.5 How does this study design tackle the management of cases that might experience weight gain after the FMT procedure? Addressing these concerns will enhance the clarity, and scientific rigor, of the study design.

This is an important ethical point. Manipulation of the gut flora could in theory lead to weight gain. The data from the 87 clinical patients who participated in the 12-month life-style change program at the medical department, UNN Harstad will be very useful as a comparison to see if there are any deviations beyond what was expected.

In the 87 patients, the average weight loss was about 2.6%, but if we looked at those who achieved more than 5% weight loss, 35% of the patients had an average weight loss of 12.5%.
The remaining 65% of the patients, on the other hand, gained an average of 2% in weight. We have a theory based on the literature that patients with a traumatic childhood have less chance of achieving weight loss with conservative treatment than those without, and we have therefore questionnaires regarding childhood trauma and mental health in the study population and will thus be able to correlate this with the weight results. This combined data will be able to provide us with information on whether there are patients in the treatment group who have a completely unexpected course.