STUDY PROTOCOL

Version 5

Fecal microbial transplantation and fiber supplementation in subjects with obesity and metabolic syndrome.

PROTOCOL VERSION DATE

October 1, 2018

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Study Summary

Title	Fecal microbial transplantation and fiber supplementation in subjects with obesity and metabolic syndrome
Short Title	FMT and fiber in metabolic syndrome
Protocol Number	Pro00076642
Phase	This is a phase II clinical trial.
Methodology	This is an exploratory, four-arm, parallel design, randomized placebo-controlled intervention study.
Study Duration	12 weeks
Study Center(s)	This is a single center trial at the University of Alberta
Objectives	The objective of this study is to determine if fecal microbial transplantation (FMT) combined with supplementation with a combined fiber supplement of resistant starch type 4, acacia gum, and soluble corn fiber has a clinically significant effect on metabolic outcomes in obese subjects with metabolic syndrome
Study Outcomes	**Primary Outcome** Changes in insulin sensitivity between the time of screening and 6 weeks following treatment. **Secondary Outcomes** • Changes in Body weight and anthropometric parameters between baseline and week 6. • Changes in HbA_1C, fasting glucose, glucose tolerance test between baseline and week 6 • Changes in fasting lipid profile between baseline and week 6 • Changes in blood pressure between baseline and week 6 • Quality of life and satiety between baseline and week 6 • Changes in serum levels of leptin, adiponectin, ghrelin, CRP, TNF-α, IL-6, LPS, LPS-binding protein and zonulin between baseline and week 6 • Changes in stool microbiota composition between baseline and week 6 • Changes in stool short chain fatty acid composition between baseline and week 6
Number of Subjects	68

Diagnosis and Main Inclusion Criteria	**Primary Diagnosis:**
	• BMI ≥ 30
	
	Key Inclusion Criteria:
	• Age 18-64 years at screening
	• Total body weight fluctuation over the last 6 months <10%
	• Fasting plasma glucose > 5.6 mmol/L **OR** HgbA1c ≥ 5.5% **OR** patients receiving an antidiabetic medication
	• At least one of the following:
	  o Fasting triglyceride ≥1.7 mmol/L OR receiving dyslipidemia medication
	  o HDL cholesterol <1.03 mmol/L in males or <1.29 mmol/L in females OR receiving dyslipidemia medication
	  o Known diagnosis of hypertension **OR** systolic blood pressure ≥130 or diastolic blood pressure ≥85 mmHg OR receiving antihypertension medication

Study Product, Dose, Route, Regimen	**FMT:** • 50 gm of screened and encapsulated single donor stool (approximately 20-30 capsules) taken by mouth on day 1 of the trial after having fasted overnight and completed a bowel prep with Pico-Salax®. • Placebo pills will contain microcrystalline cellulose **Soluble corn fiber (PROMITOR®: Tate&Lyle)** • **Women:** 4.5 gm of PROMITOR by mouth days 1-3 increased to 9 gm daily from day 4 until trial completion. • **Men:** 5.5 gm of PROMITOR by mouth days 1-3 increased to 11 gm by mouth daily from day 4 until trial completion. **Resistant Wheat Starch 4 (Fibersym®: MGP Ingredients**): • **Women:** 4.5 gm of powdered RS4 by mouth days 1-3 increased to 9 gm by mouth daily from day 4 until trial completion. • **Men:** 5.5gm of powdered RS4 by mouth days 1-3 increased to 11 gm by mouth daily from day 4 until trial completion. **Acacia Gum (Pre-Hydrated Gum Arabic: TIC GUMS**): • **Women:** 4.5 gm of powdered acacia gum by mouth days 1-3 increased to 9 gm by mouth daily from day 4 until trial completion. • **Men:** 5.5gm of powdered acacia gum by mouth days 1-3 increased to 11 gr by mouth daily from day 4 until trial completion.

Duration of administration	**FMT:** Single dose of 50gm donor stool or placebo (microcrystalline cellulose) on day 1. **Fiber**: Daily administration until completion at week 6
Reference therapy	Both FMT and Fiber will be placebo matched as reference therapy.
Statistical Methodology	Study groups with by analyzed by pair-wise comparison with evaluation of means between and across groups using paired or unpaired t-tests for continuous outcomes and chi-squared tests for dichotomous ones. Multivariable predictors of change in relevant outcomes will be identified using appropriately constructed and calibrated linear regression models for continuous outcomes or logistic regression models for dichotomous ones.

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List of Abbreviations

AE	Adverse event
ASM	Anthropometric Standardization Reference Manual
BMI	Body mass index
CEGIIR	Center of Excellence in Gastrointestinal Inflammation and Immunity Research
CRP	C reactive protein
DNA	Deoxyribonucleic acid
EQ-5D	EuroQol five dimensions questionnaire
EtOH	Ethanol
FPG	Fasting plasma glucose
FMT	Fecal microbiota transplantation
GI	Gastrointestinal
HAV	hepatitis A virus
HBV	hepatitis B virus
HBT	human biotherapy
HCV	hepatitis C virus
Hgb	hemoglobin
HIV	human immunodeficiency virus
HTLV	human T lymphotrophic virus
HbA1C	Glycated haemoglobin
HDL	High density lipoprotein
HEC	hyperinsulinemic euglycemic clamp
HOMA-IR	Homeostasis model assessment-insulin resistance
HREB	Health research ethics board
IL	Interleukin
INR	International normalizing ratio for pro-thrombin time
IR	Insulin resistance
IWQOL	Impact of weight on quality of life
LPS	Lipopolysaccharide
NIH	National Institutes of Health
OGTT	Oral glucose tolerance test
RCT	Randomized controlled trial
REDCap	Research Electronic Data Capture
RS4	Resistant wheat starch 4
SAE	Serious adverse event
SCFA	Short chain fatty acid
TG	Triglyceride
TNF	Tumor necrosis factor
U of A	University of Alberta
WHO	World Health Organization

1 Background

1.1 Investigational Agent

- Fecal microbial transplant(FMT)
- Soluble corn fiber (PROMITOR®: Tate&Lyle)
- Resistant Wheat Starch 4 (Fibersym®: MGP Ingredients):
- Acacia Gum (Pre-Hydrated Gum Arabic: TIC GUMS):
- Microcrystalline Cellulose (Microcel FG-200; Blanver Farmoquimica Ltd) (Placebo)

1.2 Preclinical Data

Obesity is characterized by gut dysbiosis and a systemic low-grade inflammation that is implicated in development of insulin resistance and cardiovascular dysfunction [1]. A role for microbial produced metabolites including short chain fatty acids (SCFA) and modified bile acids on these parameters has been described in numerous publications [2-4]. Increased SCFA in particular have been shown to beneficial in weight loss possibly through a SCFA-induced enhancement of gut barrier function, increased anti-inflammatory immune responses, and modulation of energy metabolism and production of gut hormones [5, 6].

Studies using therapies aimed at altering gut microbes with fecal transplantation or antibiotics have demonstrated links between alterations in gut microbes and host metabolic parameters [7, 8]. In a study using fecal microbial transplantation (FMT), Vrieze et al showed that obese subjects who received fecal transplants from lean donors had improved insulin sensitivity and higher levels of butyrate-producing bacteria compared to obese individuals who received autologous FMT [7]. Previous studies have shown that FMT induces changes in metabolic parameters within 6 weeks but alterations in both gut microbes and metabolic parameters are not maintained suggesting an alternative strategy is required [7, 9]. In a second study, the same group showed that vancomycin acted to decrease gut microbiota diversity, bile acid dihydroxylation and peripheral insulin sensitivity in subjects with metabolic syndrome [8]. These studies, along with numerous other animal studies, clearly indicate a role for gut microbes in the modulation of host metabolic parameters.

Recent work has highlighted a necessary role for dietary fiber in the maintenance of microbes required for human health. In addition, studies suggest an inverse relationship between systemic inflammation, metabolic disease and dietary fiber intake [10]. This may be due to the fact that dietary fiber is not digested by the host but instead is fermented by the gut microbiota into SCFA and other metabolites. Individual differences in microbial composition can determine which fiber types are fermented and to what degree. Individual microbes prefer different glycans and selective fermentation of different fibers can influence which microbial taxa proliferate [11]. In addition, the colonic microbiome exists as an ecosystem, where substantial cross-feeding occurs with metabolites produced by one organism being used by a second organism [12]. Therefore, to ensure growth of an entire ecosystem and enhance engraftment of an FMT, a variety of substrates needs to be available [13]. Resistant starch type

4 (RS4), a prebiotic supplement derived from phosphorylated wheat-based resistant starch, resists host digestion and is fermented by the gut microbiota into anti-inflammatory SCFA [14]. RS4 has been used as a supplement in both human and animal studies to improve a number of metabolic parameters and is tolerated at high doses [15-18]. Human studies have shown RS4 to improve various aspects of host metabolic function including dyslipidemia, body composition, pro-inflammatory cytokines, glycosylated hemoglobin, and to attenuate post-prandial glucose and insulin responses [15, 17, 18]. RS4 has also been shown to modulate gut microbial composition and increase levels of SCFA [17]. Increases in SCFA have been shown to improve gut barrier function, induce anti-inflammatory regulatory T-cells, impact the production of gut hormones involved in energy metabolism, glucose control, and satiety as well as altering gut-brain neural circuits to enhance metabolic benefits [5, 19-22]. Acacia gum is a water soluble polysaccharide dietary fiber that is resistant to host digestion and fermented in the distal colon resulting in the production of SCFA [23]. Soluble corn fiber is a maize-derived fiber that has a prebiotic effect in increasing numbers of bifidobacteria and increased acetate production [24]. Microcrystalline cellulose is largely resistant to microbial fermentation and is suitable for use as a placebo control [25].

Overall these findings support the use of fiber supplements in patients with metabolic syndrome in helping to manage their condition. However, in human trials involving fiber supplementation, a high degree of inter-individual variation in clinical response is seen [26, 27]. This may occur due to the difference in microbial profiles between individuals and whether an individual possesses a particular microbial taxa capable of metabolizing any specific fiber. Thus, a subject with a gut dysbiosis associated with an obese state may not possess the necessary microbes to fully utilize a fiber supplement. If so, providing the recipient with specific microbes through FMT capable of metabolizing fiber supplements may improve clinical response. This concept is supported by a previous study that showed fiber supplementation to enhance responses to FMT in patients with ulcerative colitis [28]. This theory underlines the hypothesis of our trial investigation that supplementing individuals with a combination of fibers with enhance and maintain the beneficial effects of FMT on metabolic outcomes in obese subjects with metabolic syndrome.

1.3 Risk/Benefits

Members of the team have developed and refined the research protocols for this study, including fecal donor screening and collection, preparation of frozen fecal capsules, oral FMT delivery, prebiotic supplementation and patient safety. As such the intervention poses minimal risk to the patient. However, side effects from FMT and fibers include the potential for gastrointestinal intolerance. To mitigate this possibility, specific fibers were chosen that have documented limited GI side effects.

The individual patients may derive benefit from improved metabolic parameters and decreased body weight. At the scientific level, benefits of this study will contribute to our understanding of barriers and facilitators to altering and sustaining changes in microbial communities within a human host and further elucidating an underlying interaction between metabolic syndrome and the gut. This information will be of utility in planning further large scale randomized controlled trials.

1.4 Dose Rationale

Fecal microbiome transplant (FMT) is provided in the form of oral encapsulated formulation as we have previously used in the treatment of *C. difficile*. Individuals will fast overnight and complete a bowel preparation using Pico-Salax®, an oral, routine colonoscopy preparation. This is necessary to reduce the individual's microbiota to allow for the engraftment of the donor's species. The following day (Day 1) 50grams of FMT from a single, extensively screened universal donor will be administered in 20-30 capsules taken by mouth. This donor has been with the FMT program since 2012 and has high levels of microbes capable of producing SCFA. Orally administered encapsulated FMT will ease administration and limit risks to the patient and will only be administered once throughout the trial.

RS4 (9 g women; 11 g men), soluble corn fiber (9 g women; 11 g men.), and acacia gum (9 g women; 11 g men) are provided as a powder supplement which facilitates addition to the patients' daily diet. A combined total dose of 27 grams per day for women and 33 grams per day for men will be provided for the duration of the trial. Doses on days 1-3 of the trial will be half of that to minimize any possible gastrointestinal upset. These doses are based on guidelines from the U.S Institute of Medicine for adequate intake for dietary fiber. All of these fibers are well tolerated at these doses and have minimal gastrointestinal side effects [16, 29]. RS4, corn fiber, and acacia gum will be provided to participants in powder form on a weekly basis in pre-weighed foil packets.

1.5 Trial Conduct

This trial will be conducted in compliance with the study protocol as approved by University of Alberta Health Research Ethics Board (HREB) and in keeping with the Good Clinical Practice standards. The study protocol will be strictly adhered to with no changes taking place prior to review and approval by the University of Alberta Health Research Ethics Board (HREB) unless deviations are immediately necessary to avoid imminent hazard to patients. In the latter case, changes from the protocol will be conveyed to University of Alberta Health Research Ethics Board (HREB) as early as possible.

1.6 Population

The study population will include obese adults with a BMI ≥30 with evidence of insulin resistance and metabolic syndrome.

1.7 Literature

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2 Trial Objectives

The primary objective of this pilot feasibility study is to determine if fecal microbial transplantation (FMT) combined with supplementation with prebiotic fibers has a clinically significant effect on metabolic outcomes in obese subjects with metabolic syndrome. The hypothesis is that FMT will modify the gut microbiota of the recipient to increase SCFA-producing microbes. Supplementation with prebiotic fibers will then increase SCFA production and improve insulin sensitivity due to enhanced gut barrier function and reduced systemic inflammation.

Specific aims include analyzing FMT and fiber-induced changes in host metabolic parameters including insulin sensitivity, fasting blood glucose levels, lipid profiles, and glycosylated hemoglobin levels; along with changes in anthropometric measurements; gut hormones; inflammatory markers; and hunger and satiety. Compositional and functional changes in gut microbiota will be assessed and a correlation analysis between specific microbes, microbial

functions and metabolic parameters will be carried out. The goals are to identify associations between the gut microbiome and host metabolism and to determine if prebiotic fiber therapy is effective in maintaining beneficial effects following FMT. While these types of associations cannot define causality, results gained from this clinical trial have the potential to provide mechanistic insight in addition to an understanding of how supplemental prebiotic fiber may be beneficial in control of metabolic syndrome in an obese population.

Results from this feasibility study will inform the design of a larger randomized double-blind placebo-controlled trial to provide rigorous scientific evidence necessary to invoke policy and practice changes in the management of metabolic syndrome. Further, this feasibility study will provide evidence regarding safety and any adverse outcomes due to the interventional therapy. This is critical information that will inform patient acceptance, tolerance, and compliance. The overall goal is to create a safe, well tolerated therapy based on manipulation of the gut microbiota that is an effective treatment for patients with metabolic syndrome.

3 Trial Design

3.1 Primary Study Endpoints/Secondary Endpoints

Primary Endpoint:

The primary endpoint is the change in insulin sensitivity between the time of screening and 6 weeks following treatment.

Secondary Endpoint:

- Changes in body weight and anthropometric parameters between baseline and 6 and 12 weeks
- Changes in HbA_1C, fasting glucose, and oral glucose tolerance between baseline and 6 and 12 weeks
- Changes in Fasting lipid profile between baseline and 6 and 12 weeks
- Changes in blood pressure between baseline and 6 and 12 weeks
- Effects on quality of life and satiety between baseline and 6 and 12 weeks
- Changes in serum levels of leptin, adiponectin, ghrelin, CRP, TNF-α, IL-6, LPS, and LPS-binding protein between baseline and 6 and 12 weeks
- Changes in fecal microbiota composition between baseline and 6 and 12 weeks
- Changes in stool short chain fatty acid composition between baseline and 6 and 12 weeks

3.2 Study Design/Type

This is an exploratory four-arm, parallel design, randomized placebo-controlled intervention study in obese individuals with metabolic syndrome to evaluate whether FMT from lean

donors combined with supplementation with prebiotic fiber will have a clinically significant effect on metabolic parameters. The study includes a 2-week screening/baseline period followed by a single FMT and a 6 week study period in which prebiotic fiber or placebo will be added in powdered form to the subject's normal diet for the duration of the trial (Appendix 1). A follow-up visit at 12 weeks will be done to determine if beneficial effects are maintained in the absence of ongoing fiber intake. A parallel arm design was chosen to avoid cross-over effects.

The four groups are:

1) Control (Placebo FMT and cellulose)
2) FMT only (FMT followed by cellulose)
3) Prebiotic only (Placebo FMT and prebiotic fiber)
4) FMT + prebiotic fiber

Schedule and Procedures: Subjects will attend a total of 5 clinic appointments throughout the study for anthropometric and blood pressure measurements, dietary intake, hunger and satiety, and quality of life assessment using questionnaires, and for collection of blood and fecal samples (Appendix 2). The intervention will be stopped at 6 weeks. A final follow-up visit will occur at 12 weeks for anthropometric and blood pressure measurements, and collection of blood and stool samples.

3.3 Randomization

Subjects will be randomized to one of 4 groups via computer-generated numbers and stratified by gender. Individuals will be blinded as to their group allocation to reduce bias. Randomization concealment will be protected by several levels of security, including our secure website, password protection by only those authorized to randomize and a variable blocked randomization.

3.4 Maintenance

Randomized codes will be maintained in REDCAP. Codes will be broken at the end of the trial.

3.5 Trial Treatment

Fecal microbiome transplant (FMT): 50grams of FMT from a single, universal donor will be administered in 20-30 capsules taken by mouth. These capsules will be frozen at -70^0C until date of administration on day 1 of the trial. The FMT will be given after individuals have fasted overnight and completes a bowel preparation using Pico-Salax®, a routine colonoscopy preparation. We will use only one donor to reduce variability in donor profiles unless this donor becomes unavailable at which point we have backup donors available. Placebo FMT will consist of cellulose pills.

Fiber Supplementation:

Soluble corn fiber (PROMITOR®: Tate&Lyle)

- **Women:** 4.5 gm of PROMITOR by mouth days 1-3 increased to 9 gm daily from day 4 until trial completion.
- **Men:** 5.5 gm of PROMITOR by mouth days 1-3 increased to 11 gm by mouth daily from day 4 until trial completion.

Resistant Wheat Starch 4 (Fibersym®: MGP Ingredients):

- **Women:** 4.5 gm of powdered RS4 by mouth days 1-3 increased to 9 gm by mouth daily from day 4 until trial completion.
- **Men:** 5.5gm of powdered RS4 by mouth days 1-3 increased to 11 gm by mouth daily from day 4 until trial completion.

Acacia Gum (Pre-Hydrated Gum Arabic: TIC GUMS):

- **Women:** 4.5 gm of powdered acacia gum by mouth days 1-3 increased to 9 gm by mouth daily from day 4 until trial completion.
- **Men:** 5.5gm of powdered acacia gum by mouth days 1-3 increased to 11 gm by mouth daily from day 4 until trial completion.

The product will be supplied to patients on a weekly basis in pre-weighed foil packets.

Placebo: Placebo will consist of cellulose powder (Microcrystalline cellulose:Blanver) in identical foil packets.

3.6 Duration

The study duration is 12 weeks. Subjects will be seen in the clinic at recruitment, and then at 2 and 6 weeks following FMT. Intervention will be from 1-6 weeks. A final follow-up visit will occur at 12 weeks (Appendix 1)

3.7 Discontinuation

Enrollment in the study will be discontinued if a) 3 or more subjects receiving FMT or fiber supplementation experience similar SAEs and it is determined by the study investigator that such SAE's are related to the study procedure; or b) if any one subject who receives FMT is reported to experience an SAE that had fatal or life-threatening outcome and it is concluded that such SAE could be reasonably related to FMT or fiber supplementation. .

3.8 Product Accountability

Prebiotic fibers will be obtained from the manufacturers and tracked in an electronic accountability log for the duration of the study. This log will include the date received, amount received, batch numbers, and conditions at receipt. When prebiotic fibers are dispensed to a

participant, the date and amount provided will be documented in the accountability log. This will include the date, batch number, expiration date, unique kit identifier, and amount dispensed. A balance of remaining fibers will be maintained and documented in the study's accountability log.

Prebiotic fibers will be stored in a limited-access location on the 7th Floor of Katz Group Center according to instructions received from each supplier.

4 Selection and Withdrawal of Subjects

4.1 Inclusion Criteria

- Age ≥ 18 and < 65 years at the time of screening
- BMI ≥ 30
- Total body weight fluctuation over the last 6 months less than 10%
- Fasting plasma glucose > 5.6 mmol/L **OR** HgbA1c ≥5.5% OR patients receiving an antidiabetic medication
- At least one of the following:
    - Fasting triglyceride ≥1.7 mmol/L OR receiving dyslipidemia medication
    - HDL cholesterol <1.03 mmol/L in males or <1.29 mmol/L in females OR receiving dyslipidemia medication
    - Known diagnosis of hypertension **OR** systolic blood pressure ≥130 or diastolic blood pressure ≥85 mmHg OR receiving antihypertension medication

4.2 Exclusion Criteria

- Systolic blood pressure ≥180 or diastolic blood pressure ≥110 mmHg at screening.
- Triglyceride ≥6 mmol/L.
- Acute infectious or inflammatory condition over the presiding 4 weeks.
- Current or recent use (Previous 6 months) of insulin for diabetes control.
- History of oropharyngeal or significant esophageal dysphagia, inflammatory bowel disease, colon cancer, or colonic polyps with high grade dysplasia.
- History of autoimmune conditions or chronic inflammatory condition, such as rheumatoid arthritis, chronic active hepatitis B or C, HIV, chronic pancreatitis, advanced NASH, or liver cirrhosis.
- Active malignancy.
- Active substance abuse or excessive EtOH (defined as >2 X 8oz drinks/d).

4.3 Subject Withdrawal

Subjects will be free to withdraw from the trial without prejudice to further medical care at any time. Subjects will be withdrawn under the following circumstances: consent is withdrawn or subjects are lost to follow-up; requirement for antibiotic therapy for infection; pregnancy; symptomatic intolerance of the prebiotics as manifested by non-bloody diarrhea, abdominal pain, severe abdominal bloating or any other symptom deemed likely to be caused by the prebiotic; if

any severe adverse event thought to be related to the study compound occurs. Data collected about the subject up to the time of withdrawal will remain in the trial database and be included in the data analysis.

4.4 Treatment of Subjects

All the information obtained during subject visits shall be reported in the subject's Source Document (SD). Visits are summarized in Table 1.

Screening Visit

- Obtainment of Informed Consent
- Medical History
- Hematology and Chemistry
- Pregnancy Test

Baseline Visit Week 0

- Focused physical Exam with anthropometric assessment and vital signs
- Fecal samples for microbiome analysis and levels of SCFA
- Oral glucose tolerance test
- Blood samples for levels of CRP, adipocytoquines and intestinal barrier assessment (LPS, LPBP, zonulin)
- Food Frequency Questionnaire and 24-hours recall
- Hunger and satiety questionnaire
- Gastrointestinal Tolerance questionnaire
- Quality of life questionnaire
- Fecal microbial transplant
- Dispense fiber supplements

Week 1 – telephone interview

- Telephone interview for adverse events and compliance monitoring
- Hunger and satiety questionnaire
- Quality of life questionnaire
- Gastrointestinal Tolerance questionnaire
- 24 hrs food recall

Visit at Week 2

- Office visit for Adverse Event and Compliance Monitoring
- Hunger and satiety questionnaire
- Quality of life questionnaire
- Gastrointestinal Tolerance questionnaire
- 24 hrs food recall
- Fecal samples for microbiome analysis and levels of SCFA
- Blood samples for levels of CRP, adipocytoquines and intestinal barrier assessment (LPS, LPBP, zonulin)

- Dispense fiber supplements

Week 4 – telephone interview

- Telephone interview for adverse events and compliance monitoring
- Hunger and satiety questionnaire
- Quality of life questionnaire
- Gastrointestinal Tolerance questionnaire
- 24 hrs food recall

Visit at Week 6

- Office visit for Adverse Event and Compliance Monitoring
- Anthropometric assessment and vital signs
- Hunger and satiety questionnaire
- Quality of life questionnaire
- Gastrointestinal Tolerance questionnaire
- Oral glucose tolerance test
- Fecal samples for microbiome analysis and levels of SCFA
- Blood samples for levels of CRP, adipocytoquines and intestinal barrier assessment (LPS, LPBP, zonulin)

Visit at Week 12

- Anthropometric assessment and vital signs
- Hunger and satiety questionnaire
- Quality of life questionnaire
- Gastrointestinal Tolerance questionnaire
- Fecal samples for microbiome analysis and levels of SCFA
- Blood samples for levels of CRP, adipocytoquines and intestinal barrier assessment (LPS, LPBP, zonulin)

Table 1. General schedule for assessment and intervention.

	Visit	S1	S2	0	1 (TC)	2	4 (TC)	6	12
Interview and assessment									
	Interview	X	X	X	X	X	X	X	X
	Anthropometric assessment	X	X	X				X	X
	Vital signs (BPx2)	X	X	X		X		X	X
	Consent	X	X						
Specimen sampling and Laboratory work	TSH		X *						
	CBCD	X*	X						
	Renal Function (Cr, BUN)	X*	X						
	Electrolytes	X*	X						
	LFTs: (AST, ALT, ALP, Albumin, Bilirubin, INR)	X*	X						
	Hs-CRP		X			X		X	
	Glucose homeostasis (FPG, HbA1c, Serum insulin, glucagon, c-peptide).	X*	X			X		X	X
	Fasting lipids profile.	X*	X			X		X	X
	Infectious markers: HIV, HVB, HVA, HVC.	X*	X						
	Adipocytoquines: Leptin, Adiponectin, PYY, GLP-1, TNFα, IL-6, IL-1β.		X			X		X	X
	Intestinal Barrier: Plasma level of LPS, LPBP, zonulin		X			X		X	X
	Stool Sampling: SCFA, 16S rRNA.		X			X		X	X
Investigations	75g OGTT		X					X	
	Hunger and satiety questionnaire		X		X	X	X	X	X
	Gastrointestinal Tolerance		X		X	X	X	X	X
	EQ-ED5		X		X	X	X	X	X
	Dietary Intake (24-h recalls)		X		X	X	X	X	
	Diet History Questionnaire (DHQ)		X						
	FMT			X					

X: Sample collection

***** Not to be drawn if previous result available within 3 months

4.5 Medication

Medications permitted during this study include those under the category of oral diabetic agents, statins, fibrates and antihypertensives.

Medications not permitted during this study include insulin, anti-neoplastic agents, antibiotics, anti-retrovirals, anti-virals or medications taken with the purpose of treating underling auto-immune or inflammatory disorders.

4.6 Monitoring for subject compliance

We have designed regular clinic visits, telephone calls by the study monitor, self-documentation, and returning of treatment packages to help promote compliance. Study patients will attend a total of 6 clinic appointments throughout the 8 weeks of their participation in the study for anthropometric and blood pressure measurements, dietary intake, hunger and satiety, and quality of life assessment using questionnaires, and for collection of blood and fecal samples. Patients will be asked to keep 24-hour diet record including 2 weekdays and 1 weekend per week for 2 weeks during screening and then for the duration of the trial. Patients will be offered the option to keep records using available digital devices (i.e. Myfitness pal®, Loseit®, etc) or conventional paper-based dairies. Digital or paper base records will be collected on each visit and reviewed by the research personnel. Additionally, patients will be asked to return treatment packages to provide supplementary measures of compliance.

Patients will be provided all treatment and investigations for free under a highly trained team of investigators and reimbursed 10$ per visit for parking costs to attend follow-up clinic. Finally, we anticipate that the possibility of improving glycemic, metabolic and weight control and contributing to novel treatment methods will encourage compliance amongst our patients.

5 Assessment of Efficacy

5.1 Efficacy Parameters

The following efficacy parameters will be assessed at weeks 2, 4, and 6:

- Quality of life
- Dietary intake, hunger and satiety.
- Body weight and anthropometric parameters.
- Insulin Sensitivity Assessment: HbA_1C, fasting glucose, OGTT, Fasting lipid profile
- Blood pressure
- Blood and Plasma investigations: Serum levels of leptin, adiponectin, ghrelin, CRP, TNF-α, IL-6, LPS, LPS-binding protein, zonulin
- Fecal microbiota composition

- Stool short chain fatty acid composition

5.2 Method and Timing

Efficacy parameters will assessed during clinic visits at the times denoted in Appendix 1.

Dietary Intake and Hunger and satiety assessment: Each subject will be asked to keep 24-hour diet record including 2 weekdays and 1 weekend per week for 2 weeks during screening and then for the duration of the trial. Patients will be offered the option to keep records using available digital devices (i.e. Myfitness pal®, Loseit®, etc) or conventional paper-based dairies. Digital or paper base records will be collected on each visit and reviewed by the research personnel. Hunger and satiety will be evaluated using a previously validated instrument [30].

Health-related quality of life (HRQL): Assessment of the current health related quality of life will be accomplished by conducting standardized interviews and by using the validated questionnaires EQ-5D Index [31].

Anthropometric assessment: Anthropometric assessment will be performed by trained personnel and following the recommendations made by the CDC and published in the Anthropometry procedures Manual of the National Health and Nutrition Examination Survey (available at www.cdc.gov). Body weight will be measured using a validated, calibrated bariatric scale (Scale Tronix®) and recorded to the nearest 0.1 kg. Height will be measured to the nearest 0.1 cm using a wall-mounted stadiometer. Waist and hip circumferences will be measured following the recommendations of the Anthropometric Standardization Reference Manual (ASM), the World Health Organization (WHO) guidelines and the National Institutes of Health (NIH) guidelines. [32].

Blood and plasma samples collection: Subjects will be instructed to fast overnight prior to blood sampling. Aliquots of plasma and serum will be snap frozen in liquid nitrogen and stored in a -80C freezer in the biobank.

- ***Blood and plasma investigations***: Routine laboratory investigations will be done using the AHS clinical trial lab and will include: Cell blood count and differential (CBCD), sodium (Na) potassium (K) chloride (Cl) and carbon dioxide (CO_2), creatinine (Cr), blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), albumin, C-Reactive protein (CRP), glycated Hemoglobin HbA_1C (HbA1C), fasting blood glucose (FBG), fasting Insulin levels, plasma C-peptide, fasting lipid profile, and International Normalizing Ratio for pro-thrombin time (INR). Plasma glucose levels will be determined in fresh blood samples during the 60 minutes immediately after sample collections using standardized laboratory techniques and reported as mmol/L. Serum insulin levels will be determined in fresh or frozen samples using validated Human insulin ELISA kits following standardized laboratories techniques. Levels of leptin, ghrelin, adiponectin, TNFα, IL-6, LPS (lipopolysaccharide), LPS-binding protein and zonulin will be determined using currently accepted laboratory techniques.

Insulin sensitivity assessment: Insulin resistance (IR) is considered the cornerstone of the pathophysiology of metabolic syndrome and one of the leading drivers of the association between obesity and cardiovascular outcomes. The hyperinsulinemic euglycemic clamp (HEC) constitutes the gold standard for quantification of systemic insulin sensitivity, but it is an expensive and invasive approach. As an alternative to the HEC, multiple indirect measurements of insulin resistance have been developed using less invasive techniques and have been validated in multiple clinical and research settings.

- ***Oral Glucose tolerance test (OGTT) and surrogates of insulin resistance assessment***: The oral glucose tolerance test (OGTT) is a dynamic test that reflects the efficiency of the body to dispose of glucose after an oral load. It is commonly used in clinical scenarios to diagnose glucose intolerance and diabetes. [33] After overnight fast (> 8 h), blood samples for determination of glucose and insulin concentration will be taken at -5, 0, 30, 60, and 120 min following a standard oral glucose load.
- ***Model Assessment-Insulin Resistance (HOMA-IR):*** The homeostasis Model Assessment-Insulin Resistance (HOMA-IR) is a mathematical model to predict the interaction between glucose and insulin dynamics across a range of glucose plasma levels assuming a feedback circuit between the liver and β-cells in the pancreas.
    - $HOMA - IR = \frac{\text{fasting insulin (μIU/ml)] × [fasting glucose (mmol/l)}}{22.5}$
- Anticipating a large variability in insulin sensitivities among participant of this study, logarithmic transformation may be applied (logHOMA-IR) since it is known to increase the linear correlation of this measurement and clamp estimated insulin sensitivities.[33]

Stool sample collection: Each subject will be instructed to collect and preserve at least 30 grams of stool and store at -20^{0}C in the freezer till the next clinic visit. Each subject will be supplied with stool and urine collection kits as well as written instructions. DNA will be extracted from stool samples using the QIAamp DNA Stool Mini Kit (Qiagen, CA). Microbial composition will be characterized by 16S rRNA sequencing using MiSeq Illumina technology. Read processing and quality control, chimera removal, primer trimming, and merging of pair-end reads will be done using fastx toolkit (http://hannonlab.cshl.edu/fastx_toolkit/), merge-Illumina-utils (https://github.com/merenlab/illumina-utils) and UPARSE pipeline [34]. Data will be analyzed using taxonomic based approaches including the Ribosomal Database Project Multiclassifier tool [35] and non-taxonomic based clustering algorithms for Operational Taxonomic Unit determination available in pipelines such as UPARSE. Alpha-diversity indices and β-diversity indices will be calculated in QIIME [36]. SCFA will be quantified in fecal samples by gas chromatography.

6 Assessment of Safety

6.1 Safety Parameters

Subjects will receive an information document about the study including the contact number of the principal investigator from the study coordinator. After subjects have received the full information about study participation, expected benefits and possible side effects and

inconveniences related to study participation, written consent will be obtained. The study will be explained by study investigators, and by the study coordinators. Informed consent can be obtained either by study coordinators or investigators. The obtainment of informed consent will be documented in SD. Subject must commence study within 21 days of signing consent. Therefore, the screening visit and the Week 0 visit must be scheduled within 14 days of each other so that eligible patients can be randomized and have the FMT followed by starting the fiber supplement.

6.2 Adverse Event Monitoring

Subjects will be questioned by the research coordinator at weeks 1, 2, 4, and 6 about any Severe Adverse Events. These are defined in Section 6.4. At each visit, the study coordinator will record the occurrence and severity of side effects: unacceptable flatulence, abdominal rumbling, bloating, abdominal pain or diarrhea thought to be related to the fiber supplementation.

6.3 *Serious Adverse Events (SAEs) and Safety Monitoring*

A serious adverse event is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- results in subject hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/ birth defect

The principal investigator will notify the Health Research Ethics Board (HREB) about serious adverse events via a Local Serious Adverse Events Report. This will occur within 7 days of the event. The research coordinator will notify all subjects upon their next visit to clinic.

Clinical monitoring will be provided through the University of Alberta Quality Management in Clinical Research Department (QMCR) for the duration of the trial. All serious adverse events will be reviewed by the QMCR and the study team to determine if a pattern exists which could be related to the study. If the events are deemed to be attributable to the study treatment the treatment arms will be unmasked. If it is concluded that the SAEs could be related to the study treatment, a recommendation will be made to halt enrolment in the study.

6.5 Adverse Event Follow-up

All SAEs will be recorded in the SD. For SAEs, the investigator will obtain sufficient information to determine the causality of the adverse event. Follow-up of the adverse event, after the date of therapy discontinuation, will be required if the adverse event persists. Follow-up is required until the event is resolved.

All observed or reported adverse events considered to be related to FMT or fiber supplementation will be recorded on the adverse event page(s) of the Source Document. These adverse events will be recorded in detail; onset during the study, duration, nature, severity, action taken and outcome.

Severity will be defined as:

- Mild

- Moderate
- Severe

Action taken will be defined as:
- None
- Supplementation temporarily interrupted
- Supplementation definitely stopped
- Other treatment

Outcome will be defined as:
- Resolved
- Ongoing
- Lost to follow up

In addition, abnormal findings that result in a change in study supplement dosage or in discontinuation of the supplement, or require intervention or diagnostic evaluation to assess the risk to the subject, will be recorded as adverse events.

7 Statistical Plan

7.1 Statistical Methods

Data analyses will be performed using Stata Statistical Software® Release 13.0 (College Station, TX, Stata Corporation) and [SAS/STAT] software, Version 9.2 of the SAS System for UNIX. Pair-wise comparisons between the study groups will be performed separately. Variables will be examined descriptively and graphically, including assessments of temporal trends and tests of normality. Mean change in each outcome will be calculated and compared within each study group and between study groups using paired or unpaired t-tests for continuous outcomes and chi-squared tests for dichotomous ones. Multivariable predictors of change in relevant outcomes will be identified using appropriately constructed and calibrated linear regression models for continuous outcomes or logistic regression models for dichotomous ones (i.e., change in the prevalence of a given dichotomous variable will be the dependent variable in the model). Statistical tests for treatment effects of FMT and prebiotcs on the abundance of bacterial populations and SCFA will be performed using Statistical Analysis Systems (SAS Institute Inc., Cary, NC). Data following normal distribution will be analyzed by ANOVA. Correlations between bacterial populations and SCFA and inflammation measurements will be assessed by multivariate statistical methods such as principle component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) and further confirmed by Spearman correlation tests.

All analyses related to the clinical outcome will be by intention to treat. In the case of drop-outs or missing data, where possible “a last value carried forward” approach will be used. Analyses will be conducted by a biostatistician at EPICORE Centre who is blinded to treatment allocation. Prebiotic adherence will be reported as a simple proportion of prebiotics actually taken divided by the prebiotics dispensed to form a prebiotics possession ratio – a standard method of reporting medication adherence.

7.2 Subject Population(s) for Analysis

Sample size and power calculation: Basic anthropometric and metabolic characteristics of subjects enrolled in the Edmonton Adult Bariatric Clinic 2011 and 2012 are presented in Table 1. Based on the reported baseline parameters and assuming a treatment allocation factor of 1:1, with repeated measurements design including 1 baseline and at least 3 follow-up measurements, accepting a type 1 error of 0.05 and a correlation between repeated measurements of 50%, a sample size of 15 subjects per arm will give the study a power of 80% to detect a change of 0.8 log [SD=1] in the logHOMA-IR value, or 80% power to detect a minimal difference of 1.1 [SD 1.1] in HbA1c levels between groups. Assuming a potential 10% drop-out rate, we will enroll a total of 68 subjects for this feasibility study. All randomized subjects who complete 6 weeks of follow-up will be selected for analysis.

7.3 Significance

The significance level used in this study will be 5% (0.05).

7.4 Termination Criteria

The study will be discontinued if a) 3 or more subjects receiving FMT or fiber supplementation experience similar SAEs and it is determined by the study investigator that such SAE's are related to the study procedure; or b) if any one subject who receives FMT is reported to experience an SAE that had fatal or life-threatening outcome and it is concluded that such SAE could be reasonably related to FMT. In this pilot feasibility trial there will be no interim analysis.

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8 Direct Access to Source Data/Documentation

The investigator and the University of Alberta will permit trial-related monitoring, audits, IHREB review and regulatory inspection(s) by providing direct access to source data/documentation.

9 Ethical Considerations

Our study will be carried out in keeping with the Canadian Good Clinical Practice standards. Current University of Alberta research policies and government regulations pertaining to this study will be followed at all times.

The current study protocol and any amendments made here forth will be submitted to the University of Alberta Health Research Ethics Board (HREB) for approval who will submit written approval or disapproval of conduct of the study.

Prior to enrolment, study subject will be provided consent forms describing the purpose and procedures of the trial. Said consent forms will contain adequate information from which patients will be able to make an informed decision regarding their choice to participate or not. A copy of

the consent form will be submitted in our application to the University of Alberta Health Research Ethics Board (HREB. Prior to enrolment in the trial, this consent form will need to be signed by the study subject and investigator obtaining consent from the individual.

10 Data Handling and Record Keeping

The principal investigator and her designees shall have responsibility to perform data management and statistical analysis of clinical data. As part of the responsibilities assumed by participating in the study, the investigators agree to maintain Source Documents which will serve as case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate source documentation as part of the case histories. Study records are comprised of the Source Documents and all other administrative documents, such as HREB correspondence, clinical trial material, supply shipment documents etc. Source documentation is defined as any hand-written or computer generated document that contains medical information or test results that have been collected. These may include: lab reports, clinic notes, drug disbursement log, patient sign-in sheets, patient completed questionnaires, telephone logs, etc. All draft, preliminary and pre-final iterations of a final report are also considered to be source documents, *e.g.*, fax lab reports and hard copy lab reports initial results and hard copy full, final report.

11 Finance and Insurance

This project will be financed by combined sources with the majority of funding granted from The W. Garfield Weston Foundation – *Weston Family Microbiome Initiative*. The University of Alberta proper, University of Alberta CIP program, Alberta Innovates and Canadian Institutes for Health will cover salary costs of participating researchers. Alberta Health Services has donated in-kind the use of their FMT program including donor screening and supplies for FMT encapsulation.

12 Supplements

Appendix 1: Trial Design
Appendix 2: Fiber Supplements
Appendix 3; Questionnaires

Appendix 1 Trial Design