RESEARCH ARTICLE

Fecal microbiota transplantation for the improvement of metabolism in obesity: The FMT-TRIM double-blind placebo-controlled pilot trial

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Abstract

Background
There is intense interest about whether modulating gut microbiota can impact systemic metabolism. We investigated the safety of weekly oral fecal microbiota transplantation (FMT) capsules from healthy lean donors and their ability to alter gut microbiota and improve metabolic outcomes in patients with obesity.

Methods and findings
FMT-TRIM was a 12-week double-blind randomized placebo-controlled pilot trial of oral FMT capsules performed at a single US academic medical center. Between August 2016 and April 2018, we randomized 24 adults with obesity and mild–moderate insulin resistance (homeostatic model assessment of insulin resistance [HOMA-IR] between 2.0 and 8.0) to weekly healthy lean donor FMT versus placebo capsules for 6 weeks. The primary outcome, assessed by intention to treat, was change in insulin sensitivity between 0 and 6 weeks as measured by hyperinsulinemic euglycemic clamps. Additional metabolic parameters were evaluated at 0, 6, and 12 weeks, including HbA1c, body weight, body composition by dual-energy X-ray absorptiometry, and resting energy expenditure by indirect calorimetry. Fecal samples were serially collected and evaluated via 16S V4 rRNA sequencing. Our study population was 71% female, with an average baseline BMI of 38.8 ± 6.7 kg/m² and 41.3 ± 5.1 kg/m² in the FMT and placebo groups, respectively. There were no statistically significant improvements in insulin sensitivity in the FMT group compared to the placebo group (+5% ± 12% in FMT group versus −3% ± 32% in placebo group, mean difference 9%, 95% CI −5% to 28%, p = 0.16). There were no statistically significant differences between groups for most of the other secondary metabolic outcomes, including HOMA-IR (mean difference 0.2, 95% CI −0.9 to 0.9, p = 0.96) and body composition (lean mass mean difference −0.1 kg,
95% CI −1.9 to 1.6 kg, \( p = 0.87 \); fat mass mean difference 1.2 kg, 95% CI −0.6 to 3.0 kg, \( p = 0.18 \), over the 12-week study. We observed variable engraftment of donor bacterial groups among FMT recipients, which persisted throughout the 12-week study. There were no significant differences in adverse events (AEs) (10 versus 5, \( p = 0.09 \)), and no serious AEs related to FMT. Limitations of this pilot study are the small sample size, inclusion of participants with relatively mild insulin resistance, and lack of concurrent dietary intervention.

Conclusions
Weekly administration of FMT capsules in adults with obesity results in gut microbiota engraftment in most recipients for at least 12 weeks. Despite engraftment, we did not observe clinically significant metabolic effects during the study.

Trial registration
ClinicalTrials.gov NCT02530385.

Author summary

Why was this study done?
- Animal studies show that body weight and glycemic regulation can be markedly altered by manipulation of the intestinal microbiota.
- Two prior studies in men with obesity and metabolic syndrome showed that a single nasoduodenal fecal microbiota transplantation (FMT) from lean donors led to small and transient improvements in glycemic outcomes.
- We hypothesized that repeated dosing with oral FMT capsules from lean donors could lead to lasting improvements in metabolic outcomes.

What did the researchers do and find?
- We randomized adults with obesity who were at high risk for development of type 2 diabetes to receive either weekly oral FMT capsules from healthy lean donors or placebo capsules for 6 weeks.
- Oral FMT was safe and tolerable, and we observed durable microbial shifts in most participants receiving FMT.
- We found no significant differences between groups in most glycemic outcomes, weight, or body composition over a 12-week period. There was a minor improvement in HbA1c after FMT as compared to placebo.
- Exploratory analyses suggest possible improvement in metabolism after FMT among study participants with low baseline microbiome diversity, similar to what was observed in a prior study.
What do these findings mean?

- Our results suggest that intestinal microbial manipulation by FMT capsules does not meaningfully alter human metabolism and weight in adults with obesity.

- Regional differences between study populations, differences in route of FMT administration, and differences in engrafting microbial species all might explain the overall negative findings and discordance with prior trials.

- Future studies should evaluate pre-selection of donors and recipients, and consider microbiome and lifestyle modifications concurrently.

Introduction

There has been much excitement about the potential role of the gut microbiome in influencing systemic metabolism and the development of diabetes and other cardiometabolic disorders [1,2]. The relationship between obesity, diet, metabolic diseases, and the microbiome is complex, and despite intense interest in this topic, there are few clinical studies to establish causality. The most intriguing data are derived from preclinical mouse models, which have demonstrated that genetic-, diet-, and medication-induced obesity result in microbiome shifts that confer susceptibility to obesity and negative metabolic outcomes when transferred to a new host [3–11]. Remarkably, weight gain has been shown to occur without mice switching to an obesity-inducing diet, suggesting an outsized role of the gut microbiome in dictating body weight. However, the germ-free and conventional animal models used in these studies do not directly replicate human diet, microbiome, or gastrointestinal physiology [12], and therefore these provocative results require clinical validation in human studies.

In humans, the potential relationships between obesity, metabolic disease, and the microbiome are less clear. Antibiotic exposure early in life increases later risk for obesity, presumably via undesirable alterations in the gut microbiome during childhood development [13,14]. Initial small cohort studies suggested that adults with obesity [15–17] and those with type 2 diabetes [18,19] have a different gut microbiome signature than lean controls, with decreased bacterial and/or genetic diversity. Larger cross-sectional cohorts of >1,000 patients showed mixed results, with some finding no consistent diversity or compositional differences between lean and obese adults [20] and others noting small but significant associations between microbiome and body mass index, metabolism, and body composition [21,22].

The most provocative clinical evidence supporting the metabolic potential of microbiota alterations is derived from 2 randomized clinical trials from the Netherlands. In a pilot study with 18 participants (n = 9 FMT recipients, n = 9 controls), and a subsequent larger follow-up study with 38 participants (n = 26 FMT recipients, n = 12 controls), one research group has observed that fecal microbiota transplantation (FMT) from lean donors can transiently improve peripheral insulin sensitivity in men with obesity and metabolic syndrome [23,24]. Nevertheless, these studies found reversion of the gut microbiome and insulin resistance in the FMT recipients back to baseline within 12–18 weeks after FMT administration, indicating a short-lived effect.

Prior clinical studies of FMT have typically relied on direct administration of fresh stool suspensions via upper or lower endoscopic procedures (e.g., esophagogastroduodenoscopy, colonoscopy), often with preceding gastrointestinal lavage and in the setting of pretreatment antibiotics [25]. While these procedures are effective delivery systems, their moderate
invasiveness limits considerations of repeated FMT administrations. We have pioneered a novel encapsulation technique to safely deliver oral encapsulated frozen FMT inocula in a clinical setting [26]. FMT capsules have proven to be as efficacious as endoscopically delivered FMT for recurrent *Clostridium difficile* colitis, and with better patient acceptability ratings [27]. Encapsulated FMT is now offered as standard care for recurrent *C. difficile* at our institution, and over 400 patients (including 202 formally reported [28]) have been treated without serious related adverse events (AEs).

Given the excellent safety and tolerability profile of FMT capsules, we sought to investigate whether repeated FMT administration could be a viable treatment strategy for durably modifying the gut microbiome and improving human metabolism. We therefore conducted a pilot double-blind randomized placebo-controlled trial that involved weekly administration of oral FMT capsules derived from healthy lean donors delivered to adults with obesity and mild-moderate insulin resistance. We hypothesized that weekly oral FMT would (1) safely and sustainably alter the microbiome among recipients with obesity and (2) improve metabolic endpoints, including insulin sensitivity as assessed by hyperinsulinemic euglycemic clamps.

**Methods**

**Study design**

FMT-TRIM was a 12-week double-blind randomized placebo-controlled pilot trial of encapsulated frozen FMT from healthy lean donors to adults with obesity and insulin resistance that was conducted at a single US academic medical center. Between August 25, 2016, and April 4, 2018, we recruited 24 study participants aged 25–60 years with BMI \( \geq 30 \) kg/m\(^2\) and mild to moderate insulin resistance, defined as having a homeostatic model assessment of insulin resistance (HOMA-IR) between 2.0 and 8.0. Exclusion criteria were antibiotic use in the prior 6 months, established diabetes, use of medications known to affect body weight or insulin sensitivity in the past 3 months, gastrointestinal or malabsorptive disorders, immunosuppression, and significant liver or renal disease. Information about donor screening, FMT and placebo capsule preparation, and other protocol details can be found in S1 Methods.

Study participants with obesity were randomized 1:1 to receive frozen FMT capsules or frozen placebo capsules. Randomization was performed by computer-generated random sequence in blocks of 4. Participants were not given any preparatory bowel cleansing but were instructed to fast for 4 hours prior to and 1 hour following capsule administrations. A bowel preparation was intentionally not included, for participant convenience and to assess whether the microbiome could be durably shifted without this procedure. Weekly oral capsule administrations were performed by study staff in a monitored clinical setting. At baseline (i.e., week 0), participants were administered 15 capsules on each of 2 consecutive days, followed by 15 capsules once a week for the next 5 weeks. Although there were multiple donors for the study, each FMT participant only received capsules from a single donor. At each capsule administration and study visit, study staff performed an interviewer-administered targeted assessment to assess potential AEs since the prior visit, which were graded in accordance with Common Terminology Criteria for Adverse Events (CTCAE) v4.0. Specific AEs assessed for included fever, diarrhea, nausea/vomiting, fatigue/malaise, headache, and distention/bloating/abdominal pain or discomfort. Study participants, investigators, and outcome assessors were masked to group assignment throughout the study. Study participants were asked to maintain a stable dietary and physical activity pattern throughout the 12-week study.

The trial was approved by the Partners Human Research Committee (protocol 2015P001632), and all study participants and donors provided written informed consent. The trial is registered on ClinicalTrials.gov (NCT02530385).
Metabolic outcomes

The primary outcome of insulin sensitivity was measured by insulin-stimulated glucose uptake (M value) during hyperinsulinemic euglycemic clamps at 0 and 6 weeks (details available in S1 Methods). Secondary metabolic measurements were performed at 0, 6, and 12 weeks, unless otherwise noted. Height and weight were measured in triplicate using a wall-mounted stadiometer (Harpenden, Seritex) and digital scale (Tanita BWB-800, Tanita Corporation of America), respectively. Dietary intake data were collected and analyzed using Nutrition Data System for Research software (Nutrition Coordinating Center, University of Minnesota). Body composition was measured by dual-energy X-ray absorptiometry (DXA) whole body scans (Hologic Discovery A), which provided subtotal body (i.e., total body excluding head) measurements of fat mass (kg), lean mass (kg), percent fat (%), and visceral adipose tissue (cm³). Resting energy expenditure measurement was performed at 0 and 6 weeks and estimated via indirect calorimetry using the VMAX 29 Encore Metabolic Cart (Vyaire Medical). Fasting blood was collected at 0, 6, and 12 weeks and stored at −80°C. Fasting glucose, hemoglobin A1c (HbA1c), lipids, and C-reactive protein were measured by standard clinical assays (Labcorp). Serum insulin was assessed by radioimmunoassay (Human Insulin-Specific RIA Kit, Millipore Corporation; inter-assay coefficient of variation 5.2%).

Microbiome assessments

Stool samples were collected from study participants at 0, 1, 3, 5, 6, and 12 weeks. A detailed description of microbiome sequencing, data processing, and donor stool sampling is provided in S1 Methods. Briefly, participants produced samples within 12 hours prior to each study visit and stored samples in an insulated transport container with frozen gel packs until delivered to the study staff. Donor preparations and study participant samples were characterized by 16S V4 amplicon sequencing. The set of unique 16S V4 DNA sequences, referred to as amplicon sequence variants (ASVs), was then inferred using the Dada2 algorithm. The Silva database was used for taxonomic assignments [29].

Additionally, we sequenced a subset of donor 1 FMT recipient and placebo samples using shotgun metagenomics, for finer taxonomic resolution. Baseline and week 1, 6, and 12 samples from the 3 donor 1 FMT recipients, 3 placebo participants, and donor 1 preparations (preps) were sequenced with shotgun metagenomics sequencing (whole metagenome sequencing [WMS]) using a Nextera DNA Flex Library Prep Kit on an Illumina HiSeq platform. We used the Metaphlan2 package to infer species-level taxonomic composition of all 24 WMS samples and Strainphlan (version 1.2.0 with parameter:–relaxed3) to infer strains and phylogenetic distances between strains of the same species.

Statistical analysis

Assuming a standard deviation of 30%–45% for insulin sensitivity change and a 2-sided alpha of 0.05, this pilot trial (n = 12 per group) had 80% power to detect a 40%–60% difference in insulin sensitivity between groups, allowing for a possible 15% dropout rate. Baseline characteristics were compared between groups using independent t test, Wilcoxon rank sum test, Fisher’s exact test, or chi-squared test, as appropriate. Data were analyzed according to intention-to-treat principles. Results are reported as mean ± standard deviation (SD) for normal data and median [Q1, Q3] for non-normal data. Our primary outcome was comparison of the percentage change in insulin sensitivity (M value) from 0 to 6 weeks in the FMT and placebo groups. Due to the presence of outliers for this outcome, we analyzed data using a Wilcoxon rank sum test. Prespecified secondary outcomes included changes between baseline and 12 weeks for the following measures: HOMA-IR, body weight, lean mass as assessed by DXA, and
fat mass as assessed by DXA. All other outcomes reported were exploratory. For outcomes measured at more than 2 timepoints (e.g., 0, 6, and 12 weeks), a longitudinal general linear mixed effects model (SAS PROC MIXED) with a compound symmetry covariance structure was used to compare change in secondary metabolic outcomes between the FMT and placebo groups over the 12-week study. The participant-specific intercept was considered a random effect, and time, group, and time × group interaction were considered fixed effects. We also performed a sensitivity analysis of the primary outcome (insulin sensitivity) using the linear mixed effects model with adjustment for the baseline value. Finally, for 1 study participant in the placebo group, the baseline insulin clamp was disrupted by a fire alarm; although data were deemed valid to keep in the main analysis, an additional sensitivity analysis excluding this outlier was also performed. Analyses of metabolic endpoints were performed using SAS 9.4 software (SAS Institute).

Microbiome alpha and beta diversity were assessed by applying the Shannon diversity index and UniFrac dissimilarity metrics [30] to 16S V4 DNA sequencing data as detailed in S1 Methods. For engraftment analysis, each ASV was considered in the context of participant–donor pairings. If an ASV was identified in any prep from the participant’s paired donor material and any of the participant’s post-dosing samples (week 1–12), but not in the participant’s baseline sample, the donor-specific ASV was considered an “engrafting” ASV. If an ASV was identified in a baseline participant sample, but not in any preps from the paired donor, it was considered a “participant-specific” ASV. If an ASV was observed in both the participant baseline sample and any paired donor prep, it was considered “common to participant and donor.” ASVs that were only observed in participants following dosing were labeled “newly detected.” Heatmaps displaying the dynamics of engrafting ASVs were generated using the ComplexHeatmap R package [31]. To display ASV abundances in heatmaps, ASV counts for each prep were subsampled to the same sequencing depth, a pseudocount of 1 was applied, values were transformed to relative abundances, and finally values were log10 transformed. To estimate enterotype status, we calculated the ratio of Prevotellaceae to Bacteroidaceae abundance. Family-level resolution instead of the more traditional genus-level resolution was used because the Silva taxonomy contains 15 Prevotella subgroups instead of a single genus.

The significance of correlations between changes in microbiome community composition and changes in metabolic measurements was quantified using Mantel tests with Spearman correlation coefficients [32]. Percentage change in ASV richness and diversity from baseline to the end of the dosing period between the FMT and placebo groups was evaluated using Wilcoxon rank sum tests. In exploratory post hoc analyses, we examined whether baseline microbial diversity influenced response to treatment, as previously suggested [24]. After excluding participants with baseline microbiome diversity above the baseline median (Shannon diversity index of 3.1), we replicated the longitudinal general linear mixed effects model (FMT n = 5, placebo n = 7) comparing clinical changes between the FMT and placebo groups throughout the 12-week study.

Results

Between August 2016 and April 2018, we screened 145 individuals to recruit 24 adults with obesity and mild–moderate insulin resistance to participate in this randomized controlled trial (Fig 1). At baseline, the FMT and placebo groups were well balanced in terms of age, sex, weight, and bionutritional measures (Table 1). Our study population was predominantly female, with an average BMI of 38.8 ± 6.7 kg/m² and 41.3 ± 5.1 kg/m² in the FMT and placebo groups, respectively. Of the 24 randomized participants, 23 (96%) completed the 12-week study, including all weekly supervised capsule administrations. Within the FMT group, 1
participant dropped out after week 2 due to gastrointestinal symptoms and did not attend subsequent study visits, and 1 participant missed the 6-week visit due to a family emergency but attended the 12-week visit. Four metabolically healthy lean donors (3 women, 1 man; BMI range 19.5–21.8 kg/m²) provided material for the FMT capsules that were delivered to 12 FMT recipients, with a range of 1 to 5 recipients per donor. The baseline characteristics of the donors are shown in Table A in S1 Data.
After 6 weeks of treatment with study capsules, there were nonsignificant improvements in insulin sensitivity in the FMT group as compared to the placebo group (percentage change in insulin-stimulated glucose uptake: +5% ± 12% FMT versus −3% ± 32% placebo; mean difference 9%, 95% CI −5% to 28%; \( p = 0.16 \); Fig 2). Results were similar when insulin-stimulated glucose uptake was corrected for steady-state insulin level (Fig A in S1 Data, \( p = 0.14 \)). Further-
more, sensitivity analysis using a linear mixed model with adjustment for baseline insulin sen-
sitivity yielded a similarly nonsignificant difference between groups (\( p = 0.46 \)). Exclusion of
the outlier in the placebo group led to a suggestion of improvement in insulin sensitivity in the
FMT group as compared to the placebo group that nevertheless was not statistically significant
(mean difference 14%, 95% CI −1% to 30%, \( p = 0.06 \)).

There were no differences between the FMT and placebo groups in change in HOMA-IR,
fat mass, fasting lipids, or resting energy expenditure over the 12-week study (Table 2). Body
weight was similarly unchanged throughout the study. There was a statistically significant but
clinically minor greater reduction in HbA1c at 12 weeks (mean difference −0.1%, 95% CI −0.3 to
−0.01%, \( p = 0.04 \)) and increase in C-reactive protein at 6 weeks (mean difference 1.8 mg/l,
95% CI 0.3 to 3.3, \( p = 0.02 \)) in the FMT group as compared to the placebo group (Table 2).
Insulin-Stimulated Glucose Uptake (M)

Wilcoxon, p = 0.16

% Change at 6 Weeks

Placebo FMT

Table 2. Metabolic parameters in FMT and placebo groups throughout the 12-week study.

| Characteristic          | Placebo group | FMT group | Difference between FMT and placebo groups in change from baseline (95% CI) |
|-------------------------|---------------|-----------|--------------------------------------------------------------------------|
|                         | Baseline      | 6 weeks   | 12 weeks | Baseline | 6 weeks | 12 weeks | Baseline to 6 weeks | Baseline to 12 weeks |
| Weight (kg)             | 111 ± 20      | 111 ± 20  | 111 ± 19 | 110 ± 26 | 114 ± 26 | 111 ± 27 | −0.2 (−2.4, 2.0)    | 0.2 (−2.0, 2.4)     |
| Lean mass (kg)          | 58 ± 12       | 58 ± 12   | 58 ± 11  | 60 ± 15  | 62 ± 15  | 61 ± 16  | −0.4 (−2.1, 1.4)    | −0.1 (−1.9, 1.6)    |
| Fat mass (kg)           | 53 ± 10       | 53 ± 10   | 52 ± 10  | 49 ± 13  | 51 ± 14  | 50 ± 14  | 1.1 (−0.7, 3.0)     | 1.2 (−0.6, 3.0)     |
| VAT volume (cm³)        | 998 ± 319     | 991 ± 285 | 976 ± 308| 1048 ± 368| 1107 ± 423| 982 ± 358| 19 (−76, 115)       | −52 (−147, 42)      |
| Fasting glucose (mmol/l)| 4.8 ± 0.4     | 4.8 ± 0.4 | 5.1 ± 0.6| 5.0 ± 0.7| 4.8 ± 0.7| 5.1 ± 0.6| 0.02 (−0.3, 0.4)    | −0.1 (−0.4, 0.3)    |
| HbA1c (%)               | 5.5 ± 0.3     | 5.5 ± 0.3 | 5.5 ± 0.3| 5.6 ± 0.2| 5.5 ± 0.4| 5.4 ± 0.4| −0.1 (−0.2, 0.1)    | −0.1 (−0.3, −0.01)  |
| HOMA-IR                 | 3.5 ± 1.9     | 3.4 ± 1.3 | 4.8 ± 1.7| 3.5 ± 1.4| 3.9 ± 1.4| 4.7 ± 2.0| 0.3 (−0.6, 1.3)     | −0.02 (−0.9, 0.9)   |
| Total cholesterol (mmol/l)| 5.1 ± 0.6    | 5.1 ± 1.1 | 5.2 ± 0.7| 5.5 ± 0.6| 5.2 ± 0.8| 5.2 ± 1.0| −0.3 (−0.8, 0.2)    | −0.3 (−0.8, 0.2)    |
| HDL (mmol/l)            | 1.2 ± 0.3     | 1.1 ± 0.3 | 1.1 ± 0.4| 1.3 ± 0.4| 1.3 ± 0.5| 1.3 ± 0.3| 0.04 (−0.1, 0.2)    | 0.08 (−0.01, 0.2)   |
| LDL (mmol/l)            | 3.3 ± 0.6     | 3.3 ± 1.2 | 3.2 ± 0.7| 3.3 ± 0.8| 3.0 ± 0.9| 2.9 ± 0.9| −0.2 (−0.6, 0.2)    | −0.2 (−0.6, 0.2)    |
| Triglycerides (mmol/l)  | 1.3 [1.1, 1.8]| 1.2 [1.1, 2.0]| 1.4 [1.0, 2.7]| 1.7 [1.1, 2.2]| 1.9 [1.2, 2.3]| 1.5 [1.3, 2.1]| −0.4 (−1.4, 0.5) | −0.8 (−1.7, 0.1) |
| CRP (mg/l)              | 3.5 [2.5, 7.3]| 3.0 [1.7, 5.0]| 4.6 [2.5, 6.8]| 2.9 [1.7, 5.6]| 3.5 [1.9, 5.0]| 2.9 [2.0, 4.1]| **1.8 (0.3, 3.3)** | −0.1 (−1.6, 1.3) |
| REE (kcal/day)*         | 1,503 ± 218   | 1,536 ± 241| n/a      | 1,588 ± 305| 1,705 ± 351| n/a      | 8.4 (−97, 114)     | n/a                  |
| Caloric intake (kcal/day)| 1,939 ± 463   | 2,006 ± 693| 1,689 ± 760| 2,121 ± 729| 2,236 ± 949| 2,331 ± 822| −50 (−603, 502)     | 389 (−155, 932)     |

Data are mean ± SD or median [Q1, Q3]. Mean differences between FMT and placebo groups with 95% confidence intervals were calculated for change between baseline and 6 or 12 weeks using longitudinal mixed effects modeling. Bold font indicates statistically significant differences between the FMT and placebo groups.

*REE was not measured at the 12-week study visit.

CRP, C-reactive protein; FMT, fecal microbiota transplantation; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; n/a, not available; REE, resting energy expenditure; VAT, visceral adipose tissue.

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There were subtle changes in caloric intake throughout the study, but these were not statistically different between groups (Table 2). In exploratory post hoc analyses, change in insulin-stimulated glucose uptake did not appear to differ by donor ($p = 0.88$) or by baseline demographics (sex, $p = 0.24$; ethnicity, $p = 0.70$) or BMI ($p = 1.00$) of the recipients.

Baseline microbiome analysis revealed that, with the exception of donor 1, preps from the same donor tended to cluster separately from baseline samples of participants with obesity (Fig B in S1 Data). Preps from donor 1 demonstrated distinctively high diversity, with approximately 50% more unique 16S V4 DNA sequences (ASVs) than preps from the other 3 donors (Fig 3), whereas the median diversity of the preps from the other 3 donors fell within the inter-quartile range of the baseline participant samples.

As expected, we observed temporal variability in placebo participant microbiomes, as well as some background similarity between placebo samples and donor material (Fig 4). We thus used the variability in placebo participant microbiome data to define the background level of endogenous microbiome variation in our analyses. The microbiomes of FMT recipients following dosing were more similar in composition to their paired donor material and less similar in composition to their own baseline sample, compared to placebo participants (Fig 4). These data suggest that FMT shifted recipients’ microbiomes away from their respective baseline compositions and towards donor compositions. Subdividing FMT recipients by their donor material revealed that this shift was observed among recipients of FMT from donors 1, 3, and 4 (Fig C in S1 Data). Although the microbiome of the single donor 2 FMT recipient did not exhibit a large shift towards the microbiome composition of donor 2 (Fig C in S1 Data, panel A), this recipient’s microbiome did demonstrate a potential shift away from baseline following FMT (Fig C in S1 Data, panel B).

We further defined specific 16S V4 DNA sequences as donor-specific (engrafting) ASVs if they were present in both donor material and post-dosing recipient samples but not in baseline recipient samples for donor–recipient pairs. ASVs observed in post-baseline recipient samples

Fig 3. Boxplot displaying the amplicon sequence variant (ASV) diversity (Shannon diversity index) identified in lean donor samples and baseline samples of participants with obesity.

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but not observed in paired sequenced donor material were categorized as newly detected, not
engrafting. All FMT recipients exhibited engraftment of donor-specific ASVs (Fig 5), but the
relative abundances of the engrafting ASVs were highly variable. For example, the percentage
of total reads in post-dosing recipient microbiome samples that mapped to engrafting ASVs
was notably high among donor 1 FMT recipients, with a sample median of 47%, whereas
median abundances for the other donors were around 9.5%. In the majority of FMT recipients,
total abundance of engrafting ASVs exceeded the background level of newly detected ASVs
(Fig 5). The exceptions were participants 13 and 18, for whom the majority of post-dosing
samples suggested that the rate of newly detected and engrafting ASVs did not exceed the
expected background variation [33].

Overall, our data suggest that bacterial strains from donor FMT capsules successfully
engrafted in the majority of our participants, although engraftment was markedly strongest
among donor 1 FMT recipients and may not have occurred at all in participants 13 and 18.
When occurring, engraftment persisted throughout the 12-week study, including 6 weeks after
the cessation of dosing. Participant microbiome similarity to donor material plateaued after
week 3, suggesting that dosing for more than 3 weeks does not result in additional engraft-
ment. However, the additional weeks of dosing could have contributed to maintenance of
engraftment.

Fig 4. Beta diversity boxplots displaying microbiome compositional similarity of each participant to their respective baseline or triplicate
donor preps. Microbiome similarity to baseline (a) and to donor (b) is compared between fecal microbiota transplantation (FMT) and placebo
groups. Placebo results shown in (b) reflect comparisons of all combinations of placebo participant to donor prep samples. However, for Wilcoxon
rank sum tests comparing similarities between FMT and placebo recipients, the similarity of each placebo participant sample to all donor prep
samples was first averaged. *p < 0.05; **p < 0.01.

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The engrafting ASVs from all donors represented a diverse set of enteric genera (Fig D–G in S1 Data). Strikingly, we observed that 2 Prevotella ASVs strongly engrafted in donor 1 FMT recipients, increasing the relative abundance of Prevotellaceae in donor 1 FMT recipient microbiomes by at least 8-fold post-dosing and thus shifting the enterotype signature of these participants towards a higher Prevotella abundance relative to Bacteroides (P/B ratio) (Fig H in S1 Data). No enterotype shifts were consistently observed among other recipients. Despite the evidence for engraftment, compared to placebo participants, FMT-treated participants did not
display a notable increase in microbiome diversity from baseline to week 6, suggesting that the FMT treatment did not impact participant microbiome diversity (Fig I in S1 Data).

A large percentage of ASVs were identified in both donor and baseline recipient material. It is unclear whether those ASVs represent the same bacterial strain present in both samples or whether the donor and participant microbiomes each contained different bacterial strains that could not be differentiated due to the limited taxonomic resolution provided by 16S V4 sequencing. To address this, we sequenced a subset of donor 1 FMT recipient and placebo samples using shotgun metagenomics, which can provide finer taxonomic resolution. These data recapitulated ASV engraftment results, showing that the 3 donor 1 FMT recipients had more bacterial species in common with donor material, and fewer bacterial species in common with their own baseline sample relative to the 3 placebo participants (Fig J in S1 Data). Additionally, among the bacterial species found in both baseline and post-FMT shotgun metagenomic samples, approximately 50% the bacterial strains of those species identified in weeks 6 and 12 were more phylogenetically related to strains found in the donor material than the baseline recipient sample (Fig K in S1 Data), suggesting those organisms originated from the FMT dose.

We did not observe any statistically significant correlations between changes in participant microbiome composition and changes in metabolic outcomes. Given prior studies that have shown low bacterial diversity as predictive of greater metabolic response to FMT [24], we performed exploratory subset analyses. Among participants with low baseline microbiome diversity (Table B in S1 Data), analyses suggested greater improvements in several metabolic outcomes at 12 weeks for those who received FMT (n = 4) versus placebo (n = 7), including total cholesterol (mean difference $-0.6 \text{ mmol/l}$, 95% CI $-1.0$ to $-0.1 \text{ mmol/l}$), HbA1c (mean difference $-0.2\%$, 95% CI $-0.4$ to $-0.01\%$), and fasting glucose (mean difference $-0.6 \text{ mmol/l}$, 95% CI $-1.1$ to $-0.1 \text{ mmol/l}$).

There were no serious AEs reported in either group throughout the 12-week study (Table C in S1 Data). More study participants reported at least 1 episode of diarrhea in the FMT group than in the placebo group, although this difference was not statistically significant (10 versus 5, p = 0.09). The majority of diarrheal symptoms were rated as mild, and there were no CTCAE grade 3+ AEs. Intriguingly, the only 4 diarrheal events rated as moderate occurred in 2 participants who received FMT from donor 4, one of whom dropped out of the study following their second event. Upon inspection of engrafting ASVs, no organisms stood out as the potential cause of the moderate AEs (Fig F in S1 Data) albeit several days separated the timing of events and stool collection. There were no imbalances between FMT and placebo groups in other symptoms.

**Discussion**

This double-blind randomized placebo-controlled trial was designed to test the safety of FMT capsules and their ability to alter the gut microbiome in adults with obesity, and to probe for a causal link between microbial changes and metabolism. We found that 6 weeks of FMT capsule administrations sustainably altered gut microbiome composition for the majority of participants without serious adverse effects, and absent any antibiotic pretreatment or lavage. Despite the encouraging engraftment signal, we did not find statistically significant differences between the FMT and placebo groups in insulin resistance, body weight, or most other metabolic markers in these adults with obesity and without diabetes. HbA1c modestly decreased at 12 weeks in the FMT group as compared to the placebo group, although the magnitude of improvement was small. Both metabolic and microbiome responses to FMT were highly variable, suggesting a complex host–recipient dynamic.
To date, only Nieuwdorp and colleagues have published interventional FMT trials in patients with obesity. In a small pilot trial, conducted in the Netherlands, 9 men with obesity who received endoscopically delivered FMT infusions from lean donors had significantly improved peripheral insulin sensitivity over 6 weeks as assessed by hyperinsulinenic euglycemic clamp [23]. The median level of insulin-mediated glucose uptake was approximately 73% higher at 6 weeks than at baseline, and was accompanied by increased gut microbial diversity. A larger follow-up study by the same group once again demonstrated a statistically significant increase in peripheral insulin sensitivity among 26 adults with obesity receiving endoscopic FMT infusion from lean donors, although the magnitude of improvement was more modest (approximately 12%) [24]. This follow-up study also documented a small decline in HbA1c at 6 weeks after FMT, which is similar to the minor improvement in HbA1c at 12 weeks in our trial. However, engrafting clades were different in the 2 prior studies, and the studies also documented different patterns of change in fecal short chain fatty acids and bile acids. In both prior studies, metabolic and microbiome changes were short-lived, having disappeared by 12–18 weeks after FMT infusion [23,24].

The aforementioned small studies as well as our current pilot trial are not definitive and should be considered hypothesis-generating. Indeed, one possible interpretation of our study is that the gut microbiome does not regulate human metabolism in the same dramatic manner as has been shown in preclinical studies. Alternatively, it is possible that a greater magnitude of FMT engraftment is required to effect systemic changes. Mouse models have shown that obesity phenotypes can be transferred to germ-free mice through colonization with obese mouse microbiota [4,5]. However, there is little evidence to date informing whether obese or lean phenotypes can be transferred to already colonized models or the extent to which the native microbiome needs to be replaced to induce a phenotypic change.

The closest human equivalent to colonizing a germ-free mouse would be the total replacement of an individual’s native microbiome, which to our knowledge has never been demonstrated. Strategies have been proposed to further improve FMT engraftment in clinical studies. For example, treating our study participants with broad-spectrum antibiotics prior to FMT dosing would likely have increased the ratio of donor to baseline microbes after FMT [34]. However, this approach could be associated with side effects and raise ethical and antibiotic stewardship concerns. It has been suggested that bowel cleansing might enhance FMT engraftment, but this strategy has not been rigorously studied, and bowel preps only minimally impact microbiome composition [35]. For these reasons, combined with the absence of a clear experimentally supported engraftment level target, we elected to begin testing the impact of FMTs on metabolic outcomes in human participants with the most minimally invasive approach. Indeed, we found evidence of engraftment in the majority of the FMT recipients without any gut preparation or pretreatment antibiotics. It nevertheless remains unclear if achieving a greater number or relative abundance of engrafting strains in our clinical study would have yielded positive metabolic outcomes more akin to results from germ-free mouse models.

It is helpful to contrast our pilot trial with the prior published clinical trials of FMT and metabolism. Baseline age, BMI, and HbA1c in our study were roughly comparable to those of participants in the Netherlands projects. However, microbiomes of donors and recipients can strongly vary in diversity, composition, and engraftment strength, as is evident in our study data, and not all donor material may be equally efficacious, nor all recipient microbiomes equally responsive to a microbiome therapy. Notably, our trial was conducted in the US, and we expect the recipients and donors from the US and Netherlands studies to have different microbiome compositions due to regional, race/ethnicity, and dietary differences in the study populations [36–38]. Furthermore, Nieuwdorp and colleagues enrolled men exclusively,
whereas our recipients receiving active FMT treatment were 67% women, and 3 out of our 4 donors were women. There are some data to suggest differences in the gut microbiome between men and women [39], and thus it is possible that there are sex-specific differences in donor FMT material, or altered metabolic responses to FMT among female recipients. The routes of FMT delivery (capsule versus endoscopy), type of FMT (frozen versus fresh), choice of controls (non-microbiome placebo versus autologous FMT), and antecedent gastrointestinal preparation (none versus bowel prep) differed from prior studies. These have not been important factors in studies of FMT for recurrent *C. difficile* colitis [40], although appropriate caution should be taken when extrapolating from FMT outcomes in other disease conditions.

Underlying causes of obesity and insulin resistance are multifactorial and likely vary among individuals. It is possible that only a subset of individuals may respond to alterations of the microbiome. Of note, the second Netherlands study reported that lower gut microbiota diversity among obese recipients at baseline predicted metabolic response to FMT [24]. In our exploratory analyses, we observed that FMT capsules led to possible improvements in total cholesterol, fasting glucose, and HbA1c among those with low microbiome diversity at baseline, although these results should be interpreted with caution given the small numbers of participants studied. It is also important to note that the magnitude of clinical improvement after FMT in these exploratory studies is modest. We found that donor microbial diversity was positively associated with better engraftment, although changes in microbial composition were not specifically correlated with metabolic outcomes. We used a different method for characterizing microbial communities than that used in the Netherlands studies; thus, direct comparisons between cohorts are difficult to make. Variable engraftment by donor and variable metabolic changes among FMT recipients raise the question of whether selecting donors with specific microbiome signatures or designing a targeted doseable microbial consortium could yield metabolic improvements.

In a noteworthy study, gut microbiome composition helped to predict glycemic responses to various diets [41]. A specific enterotype defined by a high abundance of *Prevotella* or a high ratio of *Prevotella* relative to *Bacteroides* (P/B ratio) [42] has also shown promise in predicting response to dietary interventions. For example, Kovatcheva-Datchary et al. observed that participants with a high abundance of *Prevotella* experienced greater improvements in glucose metabolism following fiber intervention than participants with low *Prevotella* abundance [43]. In addition, a study by Hjorth et al. found that participants with P/B ratios above 0.01 lost more weight after switching to a high-fiber healthy diet than participants with a P/B ratio below that threshold [44]. Thus, it is possible that additional selection criteria may be required to produce a microbiome therapeutic to optimize engraftment and metabolic responses, perhaps in the setting of a concurrent dietary intervention.

**Strengths and limitations**

Strengths of our study include the use of hyperinsulinemic euglycemic clamps, the gold-standard assessment of insulin sensitivity. In addition, we used a rigorous randomized study design with placebo controls and masking of group assignments. Limitations of this study include the small sample size of this pilot trial and the heterogeneous study population, and inclusion of participants with only mild insulin resistance, perhaps hampering our ability to detect improvements in insulin sensitivity. Additionally, we did not assess hepatic insulin sensitivity based upon prior studies that only found FMT effects on peripheral insulin resistance [23,24]. Although we used sophisticated microbiome analysis tools, we were mostly limited to the taxonomic resolution available from the 16S V4 region, such that roughly 25%–75% of reads in each participant’s baseline sample could not be differentiated from donor sequences.
Nevertheless, results from shotgun sequencing performed on a subset of samples were consistent with our 16S V4 data suggesting engraftment. As noted earlier, metabolic and microbiome responses were highly variable, and our study was not sufficiently powered to characterize subgroups of responders or outcomes specific to individual donors. Finally, we did not introduce a dietary intervention to this study design, and our study participants consumed typical high-fat, low-fiber “Western” diets. Given that a previous study in germ-free mice found that the obese or lean phenotype was only transmissible via FMT in the presence of a low-fat, high-fiber diet [3], it is possible that pairing gut microbiota modulation with a dietary intervention may be required to enhance metabolic response.

**Conclusion**

Repeated weekly oral FMT by an encapsulated frozen inoculum is safe and tolerable in adults with obesity, and oral FMT without “conditioning” the gut with antibiotics or a bowel cleanse can result in gut microbiota engraftment for at least 12 weeks in the majority of recipients. Despite the engraftment signal, we did not observe statistically significant changes in insulin sensitivity or most other metabolic parameters. Thus, it seems unlikely that FMT-induced microbiome compositional changes alone will be sufficient to treat or prevent metabolic disorders in humans. Future research should explore whether pre-selection of donors and/or recipients or specifically designed microbial compositions can optimize beneficial microbiota changes, and whether use of a microbiome intervention in conjunction with a dietary/exercise intervention may lead to synergistic metabolic improvements in adults with obesity.

**Supporting information**

S1 CONSORT Checklist. Completed CONSORT checklist. (DOC)

S1 Data. Supplementary tables and figures. (PDF)

S1 Methods. Supplementary methods. (DOCX)

S1 Obesity FMT Protocol. Clinical trial protocol. (DOCX)

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**References**

1. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. J Clin Invest. 2011; 121(6):2126–32. https://doi.org/10.1172/JCI58109 PMID: 21633181

2. Maruvada P, Leone V, Kaplan LM, Chang EB. The human microbiome and obesity: moving beyond associations. Cell Host Microbe. 2017; 22(5):589–99. https://doi.org/10.1016/j.chom.2017.10.005 PMID: 29247042

3. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013; 341(6150):1241214. https://doi.org/10.1126/science.1241214 PMID: 24009397

4. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; 444(7122):1027–31. https://doi.org/10.1038/nature05414 PMID: 17183312

5. Thaiss CA, Zeevi D, Levy M, Zilberman-Schapira G, Suez J, Tengeler AC, et al. Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. Cell. 2014; 159(3):514–29. https://doi.org/10.1016/j.cell.2014.09.048 PMID: 25417104

6. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014; 158(4):705–21. https://doi.org/10.1016/j.cell.2014.05.052 PMID: 25126780

7. Bahr SM, Weidemann BJ, Castro AN, Walsh JW, deLeon O, Burnett CML, et al. Risperidone-induced weight gain is mediated through shifts in the gut microbiome and suppression of energy expenditure. EBioMedicine. 2015; 2(11):1725–34. https://doi.org/10.1016/j.ebiom.2015.10.018 PMID: 26870798

8. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004; 101(4):15718–23. https://doi.org/10.1073/pnas.0407076101 PMID: 15508215

9. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut. 2013; 62(12):1787–94. https://doi.org/10.1136/gutjnl-2012-303816 PMID: 23197411

10. Liou AP, Paziuk M, Luevano JM, Machinini S, Turnbaugh PJ, Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med. 2013; 5(178):178ra41. https://doi.org/10.1126/scitranslmed.3006667 PMID: 23936013

11. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mukosy R, Cheng J, et al. Gut microbiomes of Malaysian twin pairs discordant for kwashiorcork. Science. 2013; 339(6199):548–54. https://doi.org/10.1126/science.1229900 PMID: 23936771

12. Nguyen TL, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? Dis Model Mech. 2015; 8(1):1–16. https://doi.org/10.1242/dmm.017400 PMID: 25561744
13. Bailey LC, Forrest CB, Zhang P, Richards TM, Livshits A, DeRusso PA. Association of antibiotics in infancy with early childhood obesity. JAMA Pediatr. 2014; 168(11):1063–9. https://doi.org/10.1001/jamapediatrics.2014.1539 PMID: 25265089

14. Stark CM, Susi A, Emeric J, Nylund CM. Antibiotic and acid-suppression medications during early childhood are associated with obesity. Gut. 2019; 68(1):62–9. https://doi.org/10.1136/gutjnl-2017-314971 PMID: 30377188

15. Ley RE, Turnbaugh PJ, Klein S, Gordon JL. Microbial ecology: human gut microbes associated with obesity. Nature. 2006; 444(7122):1022–3. https://doi.org/10.1038/4441022a PMID: 17183309

16. Le Chatelier E, Nielsen T, Qin J, Pritfi E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013; 500(7464):541–6. https://doi.org/10.1038/nature12506 PMID: 23985870

17. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457(7228):480–4. https://doi.org/10.1038/nature07540 PMID: 19043404

18. Karlsson FH, Tremaroli V, Nookaev I, Bergstrom G, Behre CJ, Fagerberg B, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature. 2013; 498(7452):99–103. https://doi.org/10.1038/nature12198 PMID: 23719380

19. Qin J, Li Y, Cai Z, Li S, Zhu F, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012; 490(7428):55–60. https://doi.org/10.1038/nature11450 PMID: 23023125

20. Finucane MM, Sharpdon TJ, Laurent TJ, Pollard KS. A taxonomic signature of obesity in the microbiome? Getting to the guts of the matter. PLoS ONE. 2014; 9(1):e84689. https://doi.org/10.1371/journal.pone.0084689 PMID: 24412666

21. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science. 2016; 352(6285):565–9. https://doi.org/10.1126/science.aad3369 PMID: 27126040

22. Beaumont M, Goodrich JK, Jackson MA, Yet I, Davenport ER, Vieira-Silva S, et al. Heritable components of the human fecal microbiome are associated with visceral fat. Genome Biol. 2016; 17(1):189. https://doi.org/10.1186/s13059-016-0680-9 PMID: 27666579

23. Vrieze A, Van Nood E, Holleman F, Salojarvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology. 2012; 143(4):913–6.e7. https://doi.org/10.1053/j.gastro.2012.06.031 PMID: 22728514

24. Kootte RS, Levin E, Salojarvi J, Smits LP, Hartstra AV, Udayappan SD, et al. Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. Cell Metab. 2017; 28(4):611–9.e6. https://doi.org/10.1016/j.cmet.2017.09.008 PMID: 28978426

25. Gupta S, Allen-Vercoe E, Petrof EO. Fecal microbiota transplantation: in perspective. Therap Adv Gastroenterol. 2016; 9(2):229–39. https://doi.org/10.1177/1756283X15607414 PMID: 26929784

26. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. JAMA. 2014; 312(17):1772–8. https://doi.org/10.1001/jama.2014.13875 PMID: 25323539

27. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent Clostridium difficile infection: a randomized clinical trial. JAMA. 2017; 318(20):1985–93. https://doi.org/10.1001/jama.2017.17077 PMID: 29183074

28. Youngster I, Mahabamunuge J, Systrom HK, Sauk J, Khalili H, Levin J, et al. Oral, frozen fecal microbiota transplant (FMT) capsules for recurrent Clostridium difficile infection. BMC Med. 2016; 14(1):134. https://doi.org/10.1186/s12916-016-0680-9 PMID: 27609178

29. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(Database issue):D590–6. https://doi.org/10.1093/nar/gks1219 PMID: 23719380

30. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005; 71(12):8228–35. https://doi.org/10.1128/AEM.71.12.8228-8235.2005 PMID: 16332807

31. Gu Z, Ellis R, Schlensker M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics. 2016; 32(18):2847–9. https://doi.org/10.1093/bioinformatics/btw313 PMID: 27207943

32. Legendre P, Legendre L. Numerical ecology. 3rd edition. Amsterdam: Elsevier. 2012. 1006 p.
34. Simmons S, Diao L, O’Brien E, Chafee M, Zhao J, Bernardo P, et al. Engraftment of Ser-287, an investigational microbiome therapeutic, is related to clinical remission in a placebo-controlled, double-blind randomized trial (Seres-101) in patients with active mild to moderate ulcerative colitis (UC). Gastroenterology. 2018; 154(6):S1371–2.

35. Fukuyama J, Rumke L, Sankaran K, Jeganathan P, Dethlefsen L, Relman DA, et al. Multidomain analyses of a longitudinal human microbiome intestinal cleanout perturbation experiment. PLoS Comput Biol. 2017; 13(8):e1005706. https://doi.org/10.1371/journal.pcbi.1005706 PMID: 28821012

36. Deschasaux M, Bouter KE, Prodan A, Levin E, Groen AK, Herrema H, et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. Nat Med. 2018; 24(10):1526–31. https://doi.org/10.1038/s41591-018-0160-1 PMID: 30150717

37. Vangay P, Johnson AJ, Ward TL, Al-Ghali th GA, Shields-Cutler RR, Hillmann BM, et al. US immigration westernizes the human gut microbiome. Cell. 2018; 175(4):962–72.e10. https://doi.org/10.1016/j.cell.2018.10.029 PMID: 30388453

38. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012; 486(7402):222–7. https://doi.org/10.1038/nature11053 PMID: 22699611

39. Dominian C, Sinha R, Goedert JJ, Pei Z, Yang L, Hayes RB, et al. Sex, body mass index, and dietary fiber intake influence the human gut microbiome. PLoS ONE. 2015; 10(4):e0124599. https://doi.org/10.1371/journal.pone.0124599 PMID: 25874569

40. Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, et al. Fecal microbiota transplant for relapsing Clostridium difficile infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. Clin Infect Dis. 2014; 58(11):1515–22. https://doi.org/10.1093/cid/ciu135 PMID: 24762631

41. Zeedi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized nutrition by prediction of glycemic responses. Cell. 2015; 163(5):1079–94. https://doi.org/10.1016/j.cell.2015.11.001 PMID: 26590418

42. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature. 2011; 473(7346):174–80. https://doi.org/10.1038/nature09944 PMID: 21508958

43. Kovatcheva-Datchary P, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, et al. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of Prevotella. Cell Metab. 2015; 22(6):971–82. https://doi.org/10.1016/j.cmet.2015.06.010 PMID: 26552345

44. Hjorth MF, Roager HM, Larsen TM, Poulsen SK, Licht TR, Bahl MI, et al. Pre-treatment microbial Prevotella-to-Bacteroides ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. Int J Obes (Lond). 2018; 42(3):580–3. https://doi.org/10.1038/ijo.2017.220 PMID: 28849543