Allogenic Fecal Microbiota Transplantation in Patients With Nonalcoholic Fatty Liver Disease Improves Abnormal Small Intestinal Permeability: A Randomized Control Trial

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INTRODUCTION: **Nonalcoholic fatty liver disease (NAFLD) is an obesity-related disorder that is rapidly increasing in incidence and is considered the hepatic manifestation of the metabolic syndrome. The gut microbiome plays a role in metabolism and maintaining gut barrier integrity. Studies have found differences in the microbiota between NAFLD and healthy patients and increased intestinal permeability in patients with NAFLD. Fecal microbiota transplantation (FMT) can be used to alter the gut microbiome. It was hypothesized that an FMT from a thin and healthy donor given to patients with NAFLD would improve insulin resistance (IR), hepatic proton density fat fraction (PDFF), and intestinal permeability.**

METHODS: **Twenty-one patients with NAFLD were recruited and randomized in a ratio of 3:1 to either an allogenic (n = 15) or an autologous (n = 6) FMT delivered by using an endoscope to the distal duodenum. IR was calculated by HOMA-IR, hepatic PDFF was measured by MRI, and intestinal permeability was tested using the lactulose:mannitol urine test. Additional markers of metabolic syndrome and the gut microbiota were examined. Patient visits occurred at baseline, 2, 6 weeks, and 6 months post-FMT.**

RESULTS: **There were no significant changes in HOMA-IR or hepatic PDFF in patients who received the allogenic or autologous FMT. Allogenic FMT patients with elevated small intestinal permeability (>0.025 lactulose: mannitol, n = 7) at baseline had a significant reduction 6 weeks after allogenic FMT.**

DISCUSSION: **FMT did not improve IR as measured by HOMA-IR or hepatic PDFF but did have the potential to reduce small intestinal permeability in patients with NAFLD.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/B531.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an obesity-related disorder characterized by having more than 5% fat by volume in the liver. NAFLD affects 20%–30% of North American adults and 80% of obese individuals (1). The metabolic syndrome is present in 67% of patients with NAFLD (2). It has been well-established that the gut microbiome plays a role in metabolism (3–6). Many have postulated that one of the reasons that obese individuals develop NAFLD is because of the differences in the composition of bacteria in the gut. Evidence from animal studies showed that transfer of the gut microbiota from obese mice or from obese humans into germ-free mice reproduced the obese phenotype (7). Yet, there have been numerous studies that have compared the gut microbiota of NAFLD, obese, and healthy

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individuals without finding consistent differences at the phylum or genus level (8–12).

As opposed to certain bacteria being responsible for the pathogenesis of NAFLD, bacterial metabolites may be the driving force. Butyrate is a short-chain fatty acid produced by the breakdown of fiber by a variety of bacteria in the gut. It increases the intestinal barrier integrity and reduces the amount of lipopolysaccharide that passes through the intestinal membrane. A decrease in butyrate and an increase in the amount of lipopolysaccharide passing through the intestinal membrane have been shown in mice to cause NAFLD and insulin resistance (IR) (13). Genetic susceptibility (14), hyperglycemia (15), and bacterial pathogens (16) can also increase the gut permeability. One study found that administering butyrate to mice fed a high-fat diet increased their energy expenditure and protected them from developing IR (17). A human study has also demonstrated that patients with NAFLD have significantly increased gut permeability compared with healthy controls, and there was a correlation with greater amounts of fat in the liver (18). Interventions to reduce gut permeability in humans have yet to be developed.

Given the relationship between the microbiome, NAFLD, and the metabolic syndrome, fecal microbiota transplantation (FMT) is being investigated to alter the microbiota composition of the intestine and treat some of these diseases. A study in mice demonstrated that IR and the fatty liver phenotype could be transmitted via FMT (19). A human study that administered FMT to 18 metabolic syndrome patients (9 allogenic [from a thin donor] and 9 autologous transplants) reported a significant increase in insulin sensitivity in the allogenic transplant group (26.2–45.3 μmol/kg/min) (20). Notably, the autologous transplant group did not experience a change in insulin sensitivity. The authors suggested that the improvement in insulin sensitivity was because of an increased abundance of butyrate-producing bacteria, although a subsequent study did not confirm the butyrate hypothesis but questioned the impact of the microbial acetate production (21).

We hypothesized that an FMT from a lean, healthy donor given to patients with NAFLD with metabolic syndrome would result in a decrease in IR (the primary outcome) and small intestinal permeability (a secondary outcome) both at 6 weeks post-FMT, and hepatic proton density fat fraction (PDFF) at 6 months (a secondary outcome). A pilot study of 21 subjects was carried out to test the hypothesis.

METHODS

Patient recruitment and randomization

Between June 2016 to April 2018, 21 patients with NAFLD were recruited by hepatologists in London, Ontario, Canada. This was a double-blinded randomized controlled trial. Patients were randomly assigned at a ratio of 3:1 to receive an allogenic or autologous FMT and had follow-up appointments for 6 months post-transplant. A summary of the timeline of appointments and tests performed can be found in Supplementary Table 1, Supplementary Digital Content 1, http://links.lww.com/AJG/B531.

Sample size calculation

Using the individual patient data from Vrieze et al. (20), the mean (SD) rate of glucose disappearance for patients receiving allogenic FMT infusion was estimated to be 30.7 (15.3) at baseline and 38.1 (19.2) at the 6-week follow-up. The correlation between time points was 0.91. The sample size calculation for paired sample *t* test, using a 2-sided test, power of 80%, and an alpha of 0.05, yielded a minimum sample size of 12 allogenic patients.

Patient inclusion criteria

1. Attendance at the gastroenterology/hepatology clinic with a diagnosis of NAFLD as per the AASLD criteria (22).
2. Willingness to provide informed consent.
3. Greater than18 years old.

Patient exclusion criteria

1. Type 1 or 2 diabetes requiring insulin (oral hypoglycemics were not excluded as long as there was no change in dosage for at least 3 months and no plan to adjust the dose).
2. Inability to attend follow-up visits.
3. Inability to provide informed written consent.
4. Ongoing use of antibiotics or probiotics.
5. Previous or planned bariatric surgery.
6. Presence of chronic intestinal diseases e.g., celiac disease, malabsorption, or colonic tumor.
7. Immunosuppression from transplantation, human immunodeficiency virus, cancer chemotherapy, or ongoing use of any immune-suppressive agents.
8. Pregnancy.

Donor selection

Forty-six potential donors were screened to find 3 suitable donors for this study. The methods of selection have been described elsewhere (23). Potential donors were excluded if there was any history in the patient or immediate family (i.e., parents, siblings, or children) of metabolic diseases (i.e., hypertension, hyperlipidemia, diabetes, or obesity), vascular, liver, autoimmune, or psychiatric diseases. Patients with a history of or high-risk activities to acquire infectious agents (new sexual partner, hospitalization, or travel to the tropics within 3 months) or antibiotic therapy within 3 months were excluded. Potential donors underwent a full physical examination and laboratory testing to rule out body mass index (BMI) $\geq$25 kg/m^2, hypertension, hyperlipidemia, elevated transaminases, or glycated hemoglobin (HbA1c) before donation. If they passed the medical examination, their stool, blood, and urine were tested for 30 different bacterial, viral, and protozoan agents to ensure that known transmissible diseases would not be passed along to recipients through FMT. Only 1 in 10 potential donors qualified for this study. Three donors in total were identified, and all provided fresh stools for the allogenic transplants. Donor characteristics are summarized in Supplementary Table 2, Supplementary Digital Content 1, http://links.lww.com/AJG/B531.

Fibrosis staging

Liver fibrosis stage was diagnosed using a variety of methods because various clinicians were involved with recruitment for this study. Biopsy (n = 9), FibroScan (n = 7), and MR elastrography (n = 5) were used. All patients had hepatic steatosis documented by ultrasound. Liver biopsies were analyzed by experienced hepatopathologists and fibrosis staged using the Brunt methodology (24) (F0 = no fibrosis; F1 = zone 3 pericellular/sinusoidal fibrosis, focal, or extensive; F2 = zone 3 plus focal or extensive periportal fibrosis; F3 = bridging fibrosis, focal or extensive; and F4 = cirrhosis). The method of histologic scoring used was the

NAFLD Activity Score (NAS), a validated scoring system for the evaluation of histologic changes in NAFLD (25). For FibroScan, the fibrosis stage was determined according to that established by Wong et al. (26) (F0-1 ≤ 7.0 kPa, F2 7.1–8.6 kPa, F3 8.7–10.3 kPa, and F4 ≥ 10.4 kPa). For MR elastography, the fibrosis stage was determined according to that established by Loomba et al. (27).

Fecal microbiota transplant

All patients were asked to drop off a fresh fecal sample within 72 hours of their scheduled FMT to keep them blinded as to whether they were going to receive an allogenic or autologous FMT. Whole stool was stored at 4 °C and processed immediately before transplantation. All patients were pretreated with a bowel cleanse using 3 envelopes/sachets of picosulphate preparation immediately after donating a baseline stool sample and before the FMT. To prepare the FMT material, 2 g of stool (from either the donor or autologous sample) and 125 mL of sterile saline were placed inside of a BA614/STR filter bag (Seward, Islandia, NY) and mixed by using the Stomacher 400 Circulator (Seward, Islandia, NY) at 230 rpm for 30 seconds. The filtered material was then transferred into sterile sample collection containers, then transferred to the endoscopy unit, and was used within 2 h. The FMT (allogenic or autologous) was delivered to the duodenum by using an endoscope.

Small intestinal permeability

Patients were asked to drink a solution of 5 g of lactulose (Calbiochem; EMD Millipore, Billerica, MA), 2 g of mannitol powder (BDH; VWR analytical, Mississauga, ON), 1.5 g of Kool Aid (Kraft Foods, Ingleside, ON), 100 g of sucrose, and 450 mL of tap water the evening before their baseline and 6-week appointments. The subjects were asked to collect all the urine that they passed throughout the night and morning of their appointment and store it in a urine collection bottle. This bottle was brought to the clinic; the total volume of urine was recorded and then aliquoted into 10 mL amounts. Concentrations of lactulose, mannitol, and sucrose were determined using high performance liquid chromatography (28).

Blood samples

Blood was collected from patients (fasting) at baseline, 2, 6 weeks, and 6 months post-FMT. The blood was used to examine the following: CBC, albumin, bilirubin, glucose, fasting insulin, HbA1c, nonesterified fatty acids, cholesterol, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total:HDL cholesterol ratio, triglycerides, apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (Alk Phos). Baseline testing for hepatic fibrosis was performed by liver biopsy and hepatic Magnetic resonance elastography.

Fecal sample collection

The fecal samples were collected the microbiota composition was sampled using a previously validated protocol (29). Briefly, patients collected a visibly soiled piece of toilet paper after passing a stool at baseline, 2, 7 days, 2, 6 weeks, 3, and 6 months post-transplant. The subjects placed the fecal sample in a Fisherbrand Opaque Sterile Sampling Bag (Fischer Scientific, ThermoFisher Scientific, Mississauga, ON) and brought it to their appointments. The samples were then frozen at −80 °C until DNA extraction took place.

DNA extraction

DNA from the toilet paper samples was extracted by using the DNeasy PowerSoil HTP 96 Kit (Qiagen, Toronto, Ontario, Canada), as per the manufacturer's instructions with the following modification: A centrifuge speed of 3,700 rpm for 10 minutes was used. Extracted DNA was stored at −20 °C until amplification.

DNA amplification

The BioMek 3,000 Laboratory Automation Workstation for automated PCR reagent set up was used to load 10 μL (2.3 pmol/μL) of 32 primers (16 left and 16 right) with unique barcodes into 96 well plates. Amplifications of the V4 region of the 16S ribosomal RNA gene were carried out with the primers (5′-3′) ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNxxxxxxxxGTGC CAGCMGCCGCGGTAA and (5′-3′) CGGTCT CGGCATTCCTGCTGAACCGCTCTTCCGAT CTNNNNxxxxxxxxGGACTACHVGGGTWTCTAAT (xxxxxxxx is a sample-specific nucleotide barcode, and the preceding sequence is a portion of the Illumina adapter sequence for library construction). The BioMek robot was then used to transfer 2 μL of template DNA into the primer containing 96 well plates. Then, 20 μL of Promega GoTaq Colorless Master Mix (Promega, Maddison, WI) was added to the DNA template and primers. The final plate was firmly sealed with a foil PCR plate cover. This plate was placed in the Eppendorf Mastercycler thermal cycler (Eppendorf, Mississauga, Ontario, Canada), where the lid was kept at 105 °C. An initial warm-up temperature of 95 °C was used for 2 minutes to activate the GoTaq. Afterward, the volumes underwent 25 cycles of 95 °C for 1 minute, 50 °C for 1 minute, and 72 °C for 1 minute. After completion, the temperature of the thermal cycler was held at 4 °C, and amplicons were then stored at −20 °C.

DNA sequencing and data analysis

Amplified DNA was sent to the London Regional Genomics Centre at Robarts Research Institute (Western University, London, Ontario, Canada). The samples were quantified (Quant-it, Life Technologies, Burlington, ON, Canada) and pooled at equimolar concentrations. The pooled libraries were cleaned using QIAquick (Qiagen, Germantown, MD) and then sequenced by using the MiSeq Illumina platform, with 2 × 300 bp paired-end chemistry. The reads were demultiplexed and filtered using dada2 (version 1.8) and custom R scripts written by Greg Gloor (github.com/ggloor/miseq_bin). The demultiplexed reads are available at NCBI SRA (BioProject: PRJNA557235). Any taxa with less than 3 counts in 30% of the samples were removed. Taxonomy was assigned using an RDP classifier provided by the dada2 package and trained against version 132 of the SILVA database. Diversity of the fecal microbiota was quantified based on Shannon's index and was calculated using the Vegan package (github.com/vegandevs/vegan). ALDEx2 was used to identify differentially abundant taxa between patients with abnormal permeability and normal permeability and patients with abnormal permeability at baseline and 6 weeks after FMT (30). ALDEx2 was also used to identify differentially abundant taxa between baseline and all subsequent time points for allogenic and autologous FMT recipients. An effect size cutoff of $>|2.5|$ was used.

MRI

Patients underwent an abdominal MRI (3T), including 3D chemical shift encoded MRI (31) at their baseline and 6-month appointment (conducted at the Robarts Research Institute). MRI data were analyzed to determine abdominal total volume (cm^3), abdominal subcutaneous adipose volume (cm^3), abdominal visceral volume (cm^3), and liver PDFF (PDFF, %) (32). Two patients were unable to have an MRI; 1 had a pacemaker and 1 was unable to fit into the apparatus.

Diet history questionnaire

Before their FMT and 6 weeks after their FMT, participants completed the online diet history questionnaire version 2 (DHQII) with portion sizes, which measured intake over the previous month. Downloaded nutrient data were reviewed by the study dietitian for plausibility, e.g., whether energy and nutrient intakes were likely to be physiologically possible, and if appropriate, this was checked with participants. Under-reporters were included in the analysis. Owing to the inaccuracies in estimation, all dietary data are reported to a maximum of 2 significant figures. Students *t* tests were used to compare the differences in the changes in intakes.

RESULTS

Patients were randomly assigned to either the allogenic (n = 15) or autologous (n = 6) FMT group. By chance, patients who were randomized to the autologous FMT group had less severe disease scoring and healthier levels of a variety of biochemical markers (Table 1). Histological scoring of patients with NAFLD can be found in Supplementary Table 3, Supplementary Digital Content 1, http://links.lww.com/AJG/B531. Two patients in the allogenic group and 1 patient in the autologous group were biopsy-confirmed patients with nonalcoholic steatohepatitis (NASH). The primary outcome for this study was IR, as measured by the HOMA-IR score. The Wilcoxon signed-rank test was used to compare HOMA-IR at baseline and 6 weeks post-FMT in both the allogenic and autologous groups. There was no significant decrease in the IR of patients who received an allogenic or autologous FMT (Figure 1a, b). One patient in the allogenic group had specific insulin and fasting glucose concentrations above the limit used to calculate the HOMA-IR score on both baseline and 6-week assessments. This patient was not included in the HOMA-IR analysis. Patients with NASH did not respond differently to FMT, regarding IR, although the sample size of patients with biopsy-confirmed NASH may have limited sensitivity (see Supplementary Figure 1, Supplementary Digital Content 1, http://links.lww.com/AJG/B531).

There was no significant difference in the hepatic PDFF 6 months post-transplant in patients who received an allogenic or an autologous FMT (Figure 2a, b). The Wilcoxon signed-rank test was used to compare the hepatic PDFF at baseline and 6 months post-FMT in both the allogenic and autologous groups. Two patients in the autologous group were unfit to have an MRI.

Small intestine permeability was assessed using the lactulose:mannitol urine test. Seven patients in the allogenic FMT group had elevated small intestinal permeability before FMT. After the allogenic FMT from a lean, healthy donor, all 7 patients had a decrease in their small intestinal permeability (Figure 3a), with 2 decreasing to within the normal range of permeability (defined by test values less than 0.025). There was no relationship between abnormal small intestinal permeability and fibrosis score as determined by the Kruskal-Wallis test ($P = 0.7767$) (see Supplementary Figure 2, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). There was no association of specific donors with an improvement in intestinal permeability. All 3 donors were successful at lowering small intestinal permeability in patients who had abnormal permeability. There were 7 patients in the allogenic group who had increased small intestinal permeability, 5 received an FMT from donor 1, 1 from donor 2, and 1 from donor 3. All 7 patients experienced a reduction in permeability after FMT (Figure 3a). Elevated baseline small intestinal permeability was observed in one of the patients who received an autologous FMT, and it improved to within the normal range of permeability; however, one patient in the autologous group had a normal baseline permeability which rose above normal at 6 weeks (Figure 3b). Overall, there was a significant improvement in small intestinal permeability in the allogenic group ($P = 0.018$), but not in the autologous group ($P = 0.563$) (Figure 3).

A recent study of patients with metabolic syndrome suggested that peripheral insulin sensitivity improved (>10%) only in the subset of allogenic transplants that had reduced fecal microbial diversity at baseline (although the FMT did not change the fecal microbial diversity in either group) (21). We compared allogenic insulin sensitivity responders (>10% improvement in HOMA-IR at 6 weeks), and nonresponders and did not find a difference in baseline fecal diversity between the 2 groups, although paradoxically the diversity did increase post-FMT in the nonresponder group (Figure 4). In addition, we compared allogenic intestinal permeability responders (defined as a patient that experienced an improvement in small intestinal permeability and had a baseline >0.025 lactulose:mannitol) and other allogenic patients (defined as a patient who did not initially have elevated small intestinal permeability; baseline <0.025 lactulose:mannitol). We did not find a difference in the baseline fecal microbial diversity between the 2 groups, although intestinal permeability responders experienced an increase in fecal microbial diversity who approached significance 6 weeks post-FMT ($P = 0.063$) (Figure 5). We used ALDEx2 to identify whether any particular taxa were differentially abundant in patients with abnormal permeability (>0.025 lactulose:mannitol) as compared to those with normal permeability and did not find any significant differences (an effect size cutoff of $>|2.5|$ was used) (see Supplementary Figure 3, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). We also compared the composition of fecal bacteria in patients with abnormal permeability at baseline and 6 weeks after FMT and did not find any differentially abundant taxa (see Supplementary Figure 4 and 5, Supplementary Digital Content 1, http://links.lww.com/AJG/B531).

We examined the fecal microbiota composition of Donor 1 and allogenic and autologous FMT recipients after transplant These changes were variable by individual in both the allogenic and autologous groups (Figures 6 and 7, respectively). Detailed per subject analysis was also conducted on Donor 1, and all patients who received an allogenic or autologous transplant (see Supplementary Figures 6–25, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). Two patients did not collect fecal samples at baseline and were excluded in this analysis; 1 patient from the allogenic group and 1 patient from the autologous group. Donors 2 and 3 moved away during the RCT and fecal samples for the microbiota analysis were not available from them.

Table 1. Characterization of patients at baseline

Variable	Allogenic FMT (n = 15)	Autologous FMT (n = 6)	Normal range
Age (yr)	47.6 (14.9)	57.5 (13.0)	
Sex (Female:male)	10:5	5:1	
Height (cm)	168.7 (10.2)	169.1 (7.4)	
Weight (kg)	103.6 (18.0)	107.6 (31.4)	
Waist-to-hip ratio	0.962 (0.053)	0.961 (0.048)	
BMI	36.3 (5.0)	37.4 (9.5)	18.5–25
Chemistry			
Albumin (g/L)	44.4 (2.0)	42.5 (2.3)	35–50
Bilirubin (μmol/L)	8.4 (2.0)	8.7 (4.6)	<20.5
Glucose, fasting (mmol/L)	7.3 (1.8)	7.9 (2.8)	3.5–5.8
Insulin (pmol/L)	196 (177)	166 (129)	<174
HOMA-IR	3.5 (1.3)	4.4 (2.1)	<1.7
HbA1c (%)	6.3 (0.9)	6.4 (1.0)	4–6
Lipids			
Total cholesterol (mmol/L)	4.68 (1.15)	3.53 (1.08)	<5.2
HDL cholesterol (mmol/L)	1.04 (0.25)	1.18 (0.25)	>0.9
LDL cholesterol (mmol/L)	2.68 (1.09)	1.76 (0.84)	<2.0
Total: HDL cholesterol ratio	4.8 (1.6)	3.0 (0.5)	<5.0
Triglycerides (mmol/L)	2.30 (1.43)	1.31 (0.25)	1.7
Nonesterified fatty acids (μmol/L)	562 (238)	616 (269)	720
ApoA1 (g/L)	1.59 (0.26)	1.69 (0.23)	
ApoB (g/L)	1.13 (0.35)	0.78 (0.22)	
ApoB:ApoA1 ratio	0.72 (0.21)	0.46 (0.10)	
Liver enzymes			
Alanine aminotransferase (U/L)	59 (27)	37 (7)	17–63
Aspartate aminotransferase (U/L)	38 (23)	31 (5)	14–40
Alkaline phosphatase (IU/L)	71 (19)	74 (16)	38–126
Abdominal total volume (cm^3)	17,945 (4,414)	10,747 (6,914)	
Abdominal subcutaneous adipose volume (cm^3)	11,911 (3,951)	8,021 (3,277)	
Abdominal visceral fat volume (cm^3)	6,041 (1878)	6,776 (877)	
Hepatic PDFF (%)	19.24 (8.33)	23.87 (14.56)	<5
Liver fibrosis scoring			
F0	4	3	
F1	5	1	
F2	2	0	
F3	1	0	
F4	4	2	

Data presented are the mean (SD) of patients at baseline. The Mann-Whitney nonparametric tests were used. Normal ranges are defined by the Medical Council of Canada (33).
FMT, Fecal microbiota transplant; HDL, high-density lipoprotein; PDFF, proton density fat fraction.

Several metabolic biochemical markers were examined in blood samples collected from the patients at each clinical visit. Exploratory analysis suggested that compared with baseline, patients 6 weeks post-allogenic FMT had lower concentrations of nonesterified fatty acids (NEFA) (mean decrease of 146.3 μmol/L; Supplementary Figure 26A, Supplementary Digital

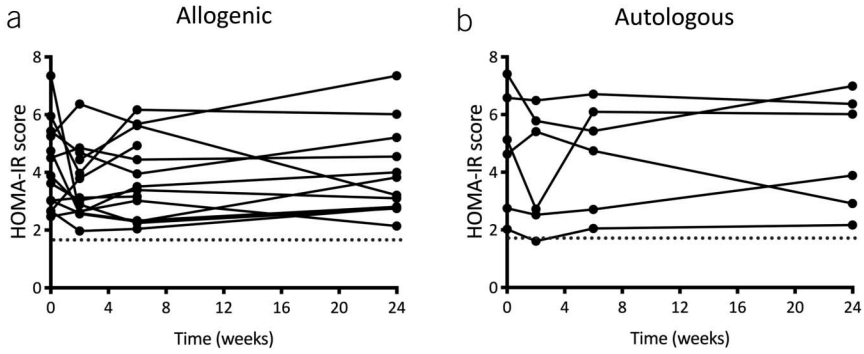

Figure 1. Insulin resistance was not significantly altered by allogenic nor autologous FMT. The HOMA-IR score was calculated using fasting glucose and insulin (specific) concentrations. The Wilcoxon signed-rank test was performed to compare the HOMA-IR scores at baseline and 6 weeks post-FMT in both the allogenic and autologous groups ($P = 0.216$ and $P = 0.688$, respectively). (**a**) Individual changes in insulin resistance in patients receiving an allogenic FMT (n = 14). Median (IQR); baseline: 3.88 (2.840–5.345), 2 weeks: 3.12 (2.56–4.57), 6 weeks: 3.45 (2.32–5.10), and 6 months: 3.52 (2.78–5.05). (**b**) Individual changes in insulin resistance in patients receiving an autologous FMT (n = 6). Median (IQR); baseline: 4.88 (2.57–6.79), 2 weeks: 4.07 (2.29–5.96), 6 weeks: 5.09 (2.55–6.25), and 6 months: 4.96 (2.73–6.53 HOMA-IR). HOMA-IR: homeostatic model assessment—insulin resistance. The dotted line represents the cutoff for a normal HOMA-IR score, <1.7. FMT, Fecal microbiota transplant; IR, insulin resistance.

Content 1, http://links.lww.com/AJG/B531). This was not observed in those receiving autologous FMT, Supplementary Figure 26B, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). Patients who received the allogenic FMT had a higher total:HDL cholesterol ratio than that of patients assigned to the autologous FMT group at baseline. A decrease in the total:HDL cholesterol ratio was observed at 6 months post-FMT in the group of patients receiving an allogenic FMT (mean decrease of 0.674; Supplementary Figure 27A, Supplementary Digital Content 1, http://links.lww.com/AJG/B531).

No changes were observed in total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides between baseline and 6 weeks post-FMT in both the allogenic and autologous groups. (see Supplementary Figures 28–31, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). No changes were observed in the concentration of fasting glucose over time in both the allogenic and autologous groups (see Supplementary Figure 32, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). No changes were observed in the ratio of ApoB:

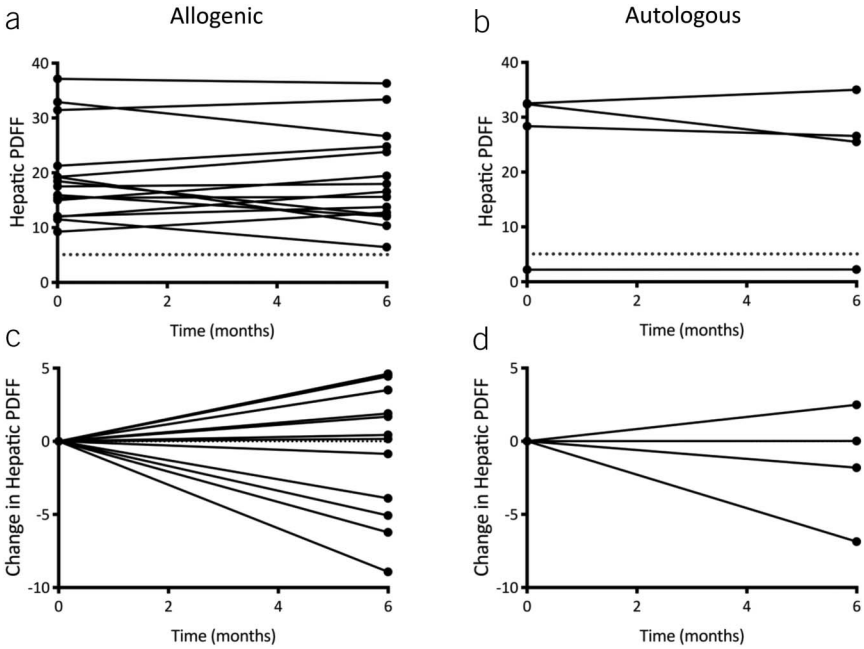

Figure 2. The hepatic PDFF in patients with NAFLD was not significantly altered by FMT. The hepatic PDFF was determined by MRI. The Wilcoxon signed-rank test was performed to compare the hepatic PDFF at baseline and 6 months post-FMT in both the allogenic and autologous groups ($P = 0.804$ and $P = 0.875$, respectively). (**a**) The hepatic PDFF over time in patients who received an allogenic FMT (n = 15). Median (IQR); baseline: 17.52% (12.1–21.29%) and 6 months: 16.6% (12.26–24.8%). (**b**) The hepatic PDFF over time in patients who received an autologous FMT (n = 4). Median (IQR); baseline 30.38% (8.759–32.48%) and 6 months: 25.52% (8.06–32.89%). (**c**) The change in the hepatic PDFF in patients who received an allogenic FMT (n = 15). (**d**) The change in the hepatic PDFF in patients who received an autologous FMT (n = 4). The dotted line represents the cutoff for a normal hepatic PDFF, < 5%. PDFF, proton density fat fraction; NAFLD, Nonalcoholic fatty liver disease.

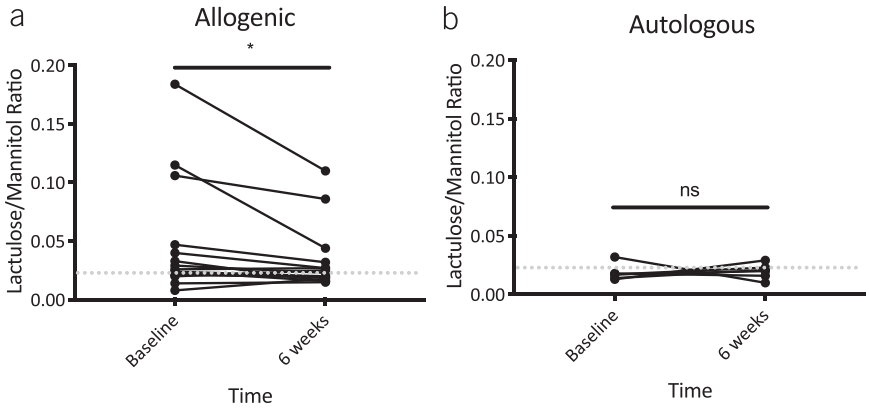

Figure 3. Patients who received an allogenic FMT from a thin and healthy donor showed improved intestinal permeability. Small intestine permeability was calculated using the lactulose:mannitol urine test. Ratios were nonzero and the Wilcoxon signed-rank test was performed to compare the lactulose:mannitol ratio at baseline and 6 weeks post-FMT in both the allogenic and autologous groups ($P = 0.018$ and $P = 0.563$, respectively). (**a**) Lactulose:mannitol ratio of patients who received an allogenic FMT (n = 15). Median (IQR); baseline: 0.026 (0.021–0.047) and 6 weeks: 0.023 (0.018–0.032). (**b**) Lactulose:mannitol ratio of patients who received an autologous FMT (n = 6). Median (IQR); baseline: 0.017 (0.0138–0.0215) and 6 weeks: 0.0205 (0.0145–0.0245). The dotted line represents the cutoff for the normal lactulose:mannitol ratio, <0.025 (35).

ApoA1 between baseline and 6 weeks post-FMT in both the allogenic and autologous groups (see Supplementary Figure 33, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). Weight, waist-to-hip ratio, and BMI were measured and calculated at each clinical visit (baseline, 2 weeks, 6 weeks, and 6 months post-FMT). Patients did not experience a change in their weight, waist-to-hip ratio, or BMI after an allogenic or an autologous FMT (see Supplementary Figures 34–36, Supplementary Digital Content 1, http://links.lww.com/AJG/B531). Seventeen patients completed the online Diet History Questionnaire Version 2 (DHQII) at baseline and at 6 weeks post-FMT (11 allogenic and 6 autologous). No changes in patients' caloric or nutrient intakes were detected (see Supplementary Figure 37, Supplementary Digital Content 1, http://links.lww.com/AJG/B531).

DISCUSSION

To our knowledge, this is the first reported clinical trial of FMT in patients with NAFLD (Clinical Trial Gov Number: NCT02496390). These data demonstrated that FMT from lean healthy donors failed to improve insulin sensitivity (Figure 1), the primary outcome. FMT also did not improve the percentage of fat in the liver on MRI at 6 months (Figure 2), a secondary outcome, but did improve intestinal permeability at 6 weeks (Figure 3).

The lack of improvement of insulin sensitivity stands in contrast to the findings of Vrieze et al. (20) in patients with metabolic syndrome where insulin sensitivity improved with allogenic transplants (n = 9 allogenic transplants). Our study of 15 allogenic patients did not demonstrate this finding; this discrepancy could be due to either technical or biological factors. Technical factors of relevance include the metric of insulin sensitivity and the study sample size. Past studies used an insulin clamp method to identify insulin sensitivity, whereas this study used a HOMA-IR score for the same purpose (20,21). Insulin clamp technology is not widely available, including at any site in Ontario. The HOMA-IR score has been shown to have excellent correlation with the insulin clamp technique (34). Furthermore, HOMA-IR is reflective of both hepatic insulin and peripheral insulin sensitivities (34). Past studies noted an improvement in peripheral insulin sensitivity ($P < 0.05$) that was modest and did not persist, but not in hepatic insulin sensitivity (20,21). By contrast, the present study had a larger number of patients receiving allogenic FMT than in the original study by Vrieze et al. (20) and a smaller number of patients receiving autologous FMT. Although the small number of autologous samples likely did not provide sufficient statistical power to detect changes in the autologous group, our primary hypothesis (improved insulin sensitivity in allogenic FMT patients) relied solely on baseline vs 6-week comparison within the allogenic group, and therefore, sample size likely does not explain the discrepancy between this study and the study of Vrieze et al. (20). Changes in HOMA-IR were not reported in previous studies (20,21). It is possible that a small improvement in peripheral, but not hepatic insulin, sensitivity may not have been adequately reflected in the HOMA-IR measure. The lack of change in the HOMA-IR raises a concern regarding the clinical impact of the small metabolic change in peripheral insulin sensitivity seen previously.

The improvement in small intestinal permeability associated with allogenic FMT was encouraging, and this is the first study to show an improvement in small intestinal permeability in NAFLD and metabolic syndrome patients after FMT. A strength of this study was the use of the gold standard lactulose/mannitol ratio as the measure of intestinal permeability. Mannitol absorption is proportional to small bowel intestinal surface area (with low values seen in conditions such as celiac disease) and lactulose has a molecular weight which normally prevents significant absorption (28). Therefore, an increase in the ratio of urinary excretion reflects increased intestinal permeability to large molecules. It has been hypothesized that increased gut permeability is a central mechanism of gut microbiome–related autoimmune diseases (such as systemic lupus and type 1 diabetes), inflammatory bowel disease, systemic inflammation and infection, metabolic syndrome, and NAFLD (35). To our knowledge, this is the first study to demonstrate that manipulation of the microbiome is associated with an improvement in intestinal permeability in patients with a syndrome which has been associated with intestinal microbiome changes, other than *C. difficile* infection. This raises the possibility of FMT or other microbiome-altering techniques being able to prevent complications of increased intestinal permeability (35). In

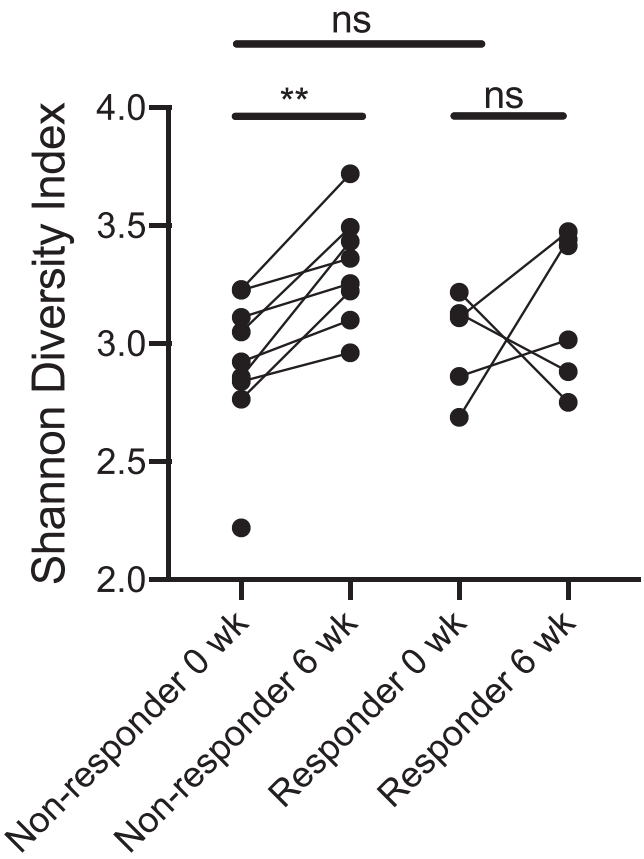

Figure 4. Fecal bacterial diversity is not related to metabolic response. Shannon diversity index at baseline and 6 weeks after allogenic FMT is shown. A responder was characterized by having >10% reduction in HOMA-IR, a nonresponder was characterized by having <10% reduction in HOMA-IR. The Mann-Whitney *t* test was used to compare the Shannon diversity at baseline in the responder (n = 6) and nonresponder groups (n = 9) ($P = 0.797$). The Wilcoxon signed-rank test was used to compare Shannon diversity index at baseline and 6 weeks post-FMT in the nonresponder and responder groups ($P = 0.0078$, $P = 0.813$, respectively). One patient in the nonresponder group did not collect a 6-week sample. IR, insulin resistance.

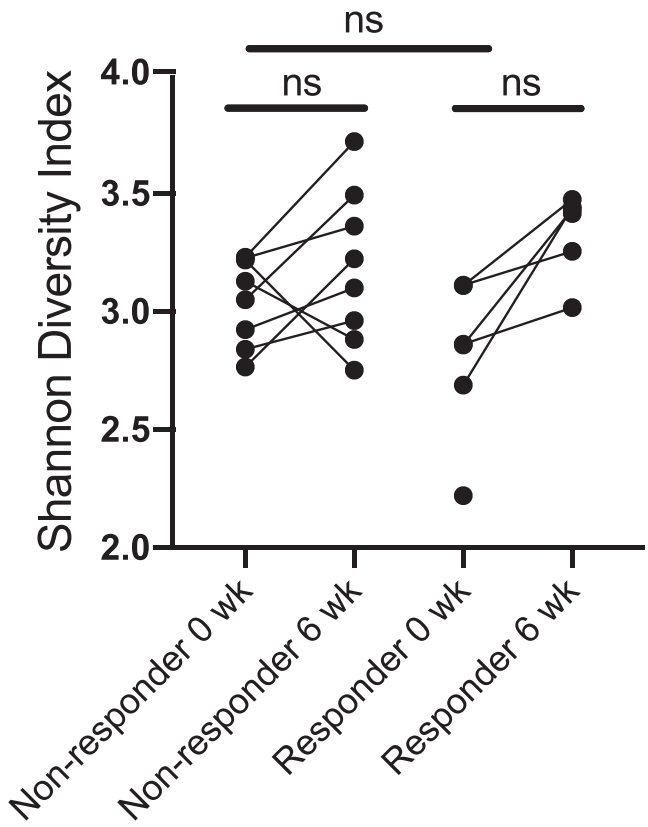

Figure 5. Fecal bacterial diversity and improvement in small intestinal permeability. The Shannon diversity index at baseline and 6 weeks after allogenic FMT is shown. A responder was characterized by having an initial lactulose:mannitol reading above 0.025 and having a reduction in this ratio at 6 weeks after allogenic FMT. The Mann-Whitney *t* test was used to compare the Shannon diversity index at baseline in the responder (n = 7) and nonresponder groups (n = 8) ($P = 0.142$). The Wilcoxon signed-rank test was used to compare the Shannon diversity index at baseline and 6 weeks after FMT in the nonresponder and responder groups with a trend toward increasing diversity seen in the responder group ($P = 0.383$, $P = 0.063$, respectively). One patient in the responder group did not collect a 6-week sample.

the future, screening patients for elevated gut permeability may be a method to select patients likely to benefit from FMT.

This is the first FMT study to monitor changes in hepatic PDFF longitudinally after FMT. No improvement in the hepatic PDFF was detected on MRI testing at 6 months after FMT. This may be because of changes in the microbiome associated with allogenic FMT not persisting for as long as 6 months. The reason for choosing this time point was to detect a meaningful change in hepatic PDFF. It is notable, however, that at 6 weeks post-FMT the NEFA (see Supplementary Figure 26, Supplementary Digital Content 1, http://links.lww.com/AJG/B531) and at 6 months post-FMT the total cholesterol: HDL cholesterol ratio (see Supplementary Figure 27, Supplementary Digital Content 1, http://links.lww.com/AJG/B531) improved in the allogenic group.

There have been very limited data assessing long-term changes in the gut microbiome after a single FMT in patients who are transplanted for a condition other than *C. difficile* (36,37). Arguably, the microbiome alterations are more easily obtained in patients with *C. difficile* infection where the microbiota diversity and richness at baseline are extremely low (38). It may be a key reason for the sustainability of the transplant in that condition (36,37). On the other hand, repeated FMT for other conditions may require repetitive interventions to prevent reversion of the microbiome to baseline. This may explain why persistent use of a probiotic led to the improvement of hepatic PDFF at 6 months (39), but the single FMT in this study failed to do so. It is also notable that although our study did not discern changes in specific taxa within the fecal microbiota with allogenic transplant, we did however observe a trend toward an increase in the fecal microbiota diversity in patients who had improvement in intestinal permeability (Figure 5). The lack of changes in specific bacterial taxa may reflect that the FMT was administered into the duodenum, but microbiome analysis was limited to stool specimens. Analysis of the stool may not reflect changes in the microbiome of the small bowel or proximal colon. In fact, a recent FMT study which used duodenal administration in metabolic syndrome noted that this phenomenon changes in the small intestinal bacterial taxa which were not reflected by changes in the specific fecal bacterial taxa and also not associated with a change in fecal microbial diversity (21). Alternative combination

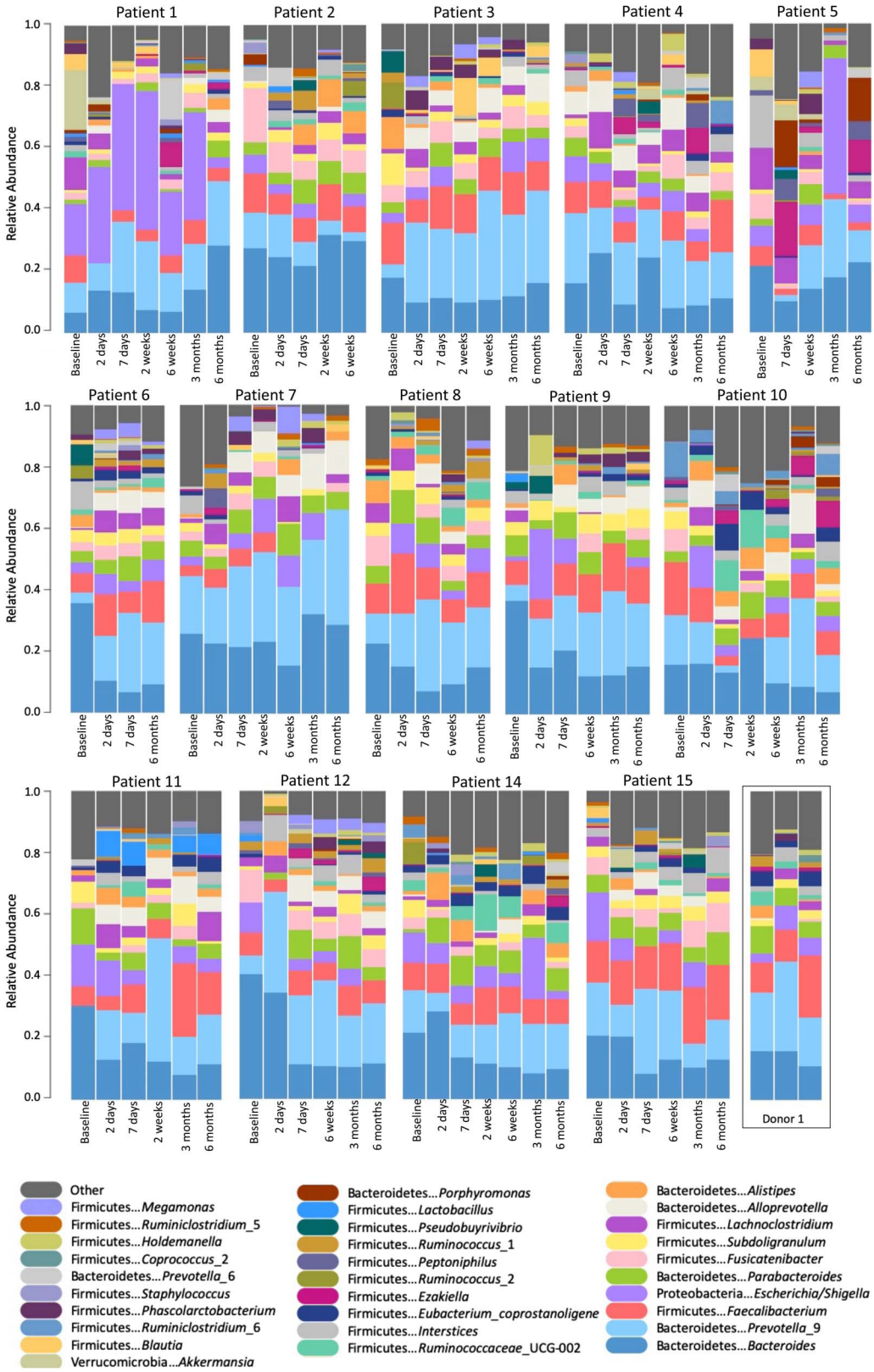

Figure 6. Fecal microbiota composition of allogenic FMT transplant recipients over 6 months. All patients except for patients 2, 10, and 11 received an allogenic FMT from donor 1. Toilet paper samples were collected from patients at baseline, 2 days, 7 days, 2 weeks, 6 weeks, 3 months, and 6 months post-FMT. The DNA was extracted, and the V4 region of the 16S rRNA gene was amplified and sent for Illumina MiSeq next-generation sequencing. The resulting reads were used to generate barplots in base R (version 3.5.3). Each column represents the fecal microbiota composition at one time point.

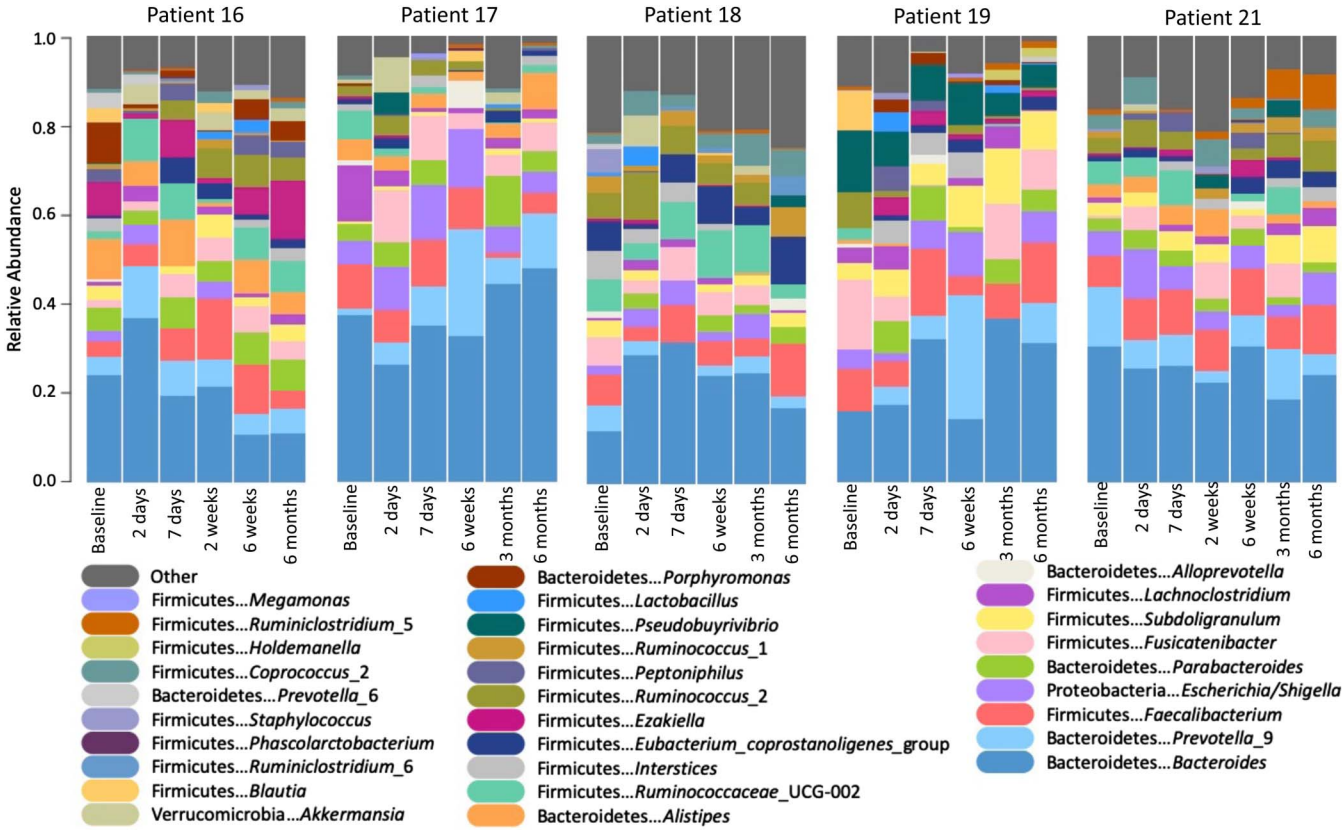

Figure 7. Fecal microbiota composition of autologous FMT transplant recipients over 6 months. Toilet paper samples were collected from patients at baseline, 2, 7 days, 2, 6 weeks, 3, and 6 months post-FMT. The DNA was extracted, and the V4 region of the 16S rRNA gene was amplified and sent for Illumina MiSeq next-generation sequencing. The resulting reads were used to generate barplots in base R (version 3.5.3). Each column represents the fecal microbiota composition at one time point.

approaches for FMT administration, such as pretreatment with antibiotics or coadministration via the colonic and duodenal route, may need to be explored. Another hypothesis would be that the changes we observed in the small intestinal permeability of the allogenic group were not dependent on specific bacterial engraftment. Sterile fecal filtrates have been shown to successfully treat patients with *C. difficile* infection (40). This may be on the basis of bacterial metabolites or possibly bacteriophages. Further studies to clarify these hypotheses are warranted.

Limitations of this study include that by random chance the autologous and allogenic groups differed regarding fibrosis staging, intestinal permeability, and biochemical data. This was likely because of the small sample size of this study. In addition, because fibrosis staging was performed before enrollment to the study as part of routine care, 3 different methods of staging fibrosis were used and more than 1 pathologist was involved in the evaluation of biopsies; this may have resulted in interobserver variability regarding NASH diagnosis, grading activity, and staging fibrosis. However, our outcome measures did not involve comparing pre- and post-treatment histological findings, so this would not affect the study outcome. A single radiologist read all MRIs for hepatic fat content both pre- and post-treatment. This adds to the reliability of our secondary outcome of PDFF.

In conclusion, duodenoscopy administered FMT did not improve insulin sensitivity (measured via HOMA-IR) or hepatic PDFF in patients with NAFLD but did contribute to the repair of intestinal permeability. The use of FMT warrants further investigation to modulate diseases associated with increased intestinal permeability including NAFLD.

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CONFLICTS OF INTEREST

Guarantor of article: Michael Silverman, MD.

Specific author contributions: L.C.: Study design, Microbiome analysis, drafting the manuscript. A.R.: Study design, performance of FMT's. S.N.P.: Study design, FMT preparation, and donor screening. M.B.: Study design, patient assessment, and review of the manuscript. J.S.: Microbiome analysis and review of the manuscript. K.Q.: Patient assessment and review of the manuscript. I.H.: Study design and review of the manuscript. R.H.: Study design and review of the manuscript. T.J.: Study design and review of manuscript. J.M.: Study design, review of

manuscript, and performance of permeability assays. B.U.: Study design and review of the manuscript. R.H.: Study design, and analysis of the Food Questionnaire results. C.M.: Study design, review of the manuscript, and analysis of the PDFF data. K.S.: Study design and review of the manuscript. G.R.: Study design and review of the manuscript. J.P.B.: Study design, review of the manuscript, and microbiome analysis. M.S.: Study design and supervision and manuscript writing.

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Potential competing interests: None to declare.

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Study Highlights

WHAT IS KNOWN

- ✓ The gut microbiome is altered in obesity- and metabolic-related diseases.
- ✓ Fecal microbiota transplantation has the potential to alter the gut microbiome and confer health benefits at distant and local sites in the body.

WHAT IS NEW HERE

- ✓ Fecal microbiota transplantation improved elevated small intestinal permeability.

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