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CLINICAL AT

Effects of Diet-Modulated Autologous Fecal Microbiota Transplantation on Weight Regain

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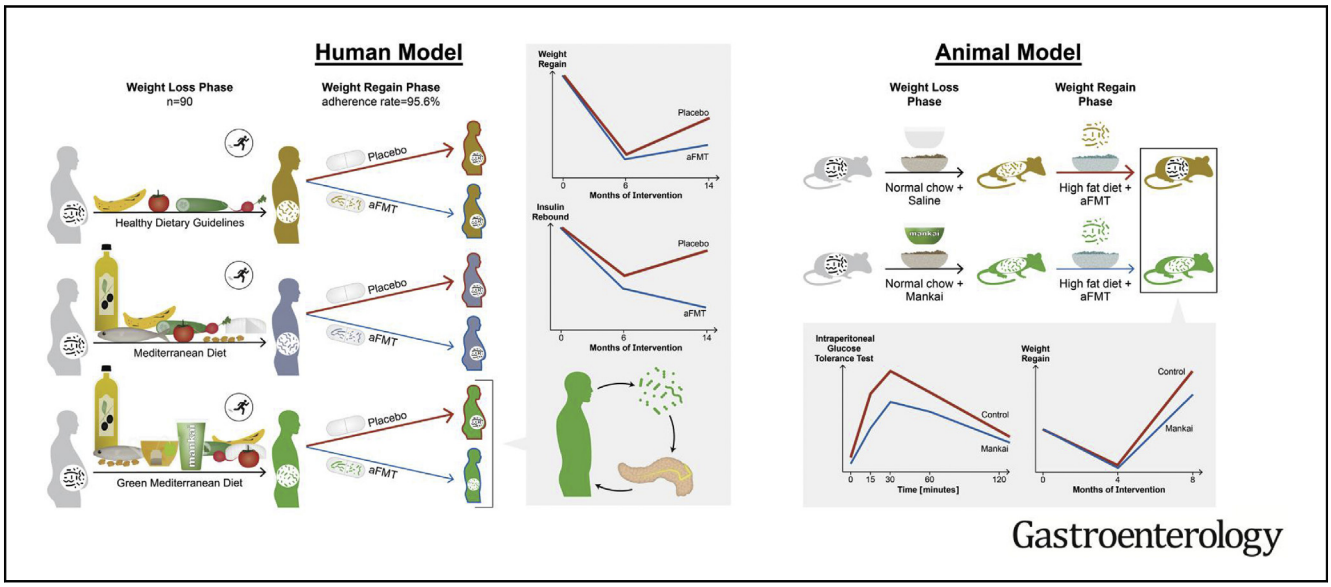

See Covering the Cover synopsis on page 1; See editorial on page 17.

BACKGROUND & AIMS: We evaluated the efficacy and safety of diet-modulated autologous fecal microbiota transplantation (aFMT) for treatment of weight regain after the weight-loss phase. **METHODS:** In the DIRECT PLUS (Dietary Intervention Randomized Controlled Trial Polyphenols-Unprocessed) weight-loss trial (May 2017 through July 2018), abdominally obese or dyslipidemic participants in Israel were randomly assigned to healthy dietary guidelines, Mediterranean diet, and green-Mediterranean diet weight-loss groups. All groups received free gym membership and physical activity guidelines. Both isocaloric Mediterranean groups consumed 28 g/d walnuts (+440 mg/d polyphenols provided). The green-Mediterranean dieters also consumed green tea (3–4 cups/d) and a *Wolffia globosa* (Mankai strain, 100 g/d) green shake (+800 mg/d polyphenols provided). After 6 months (weight-loss phase), 90 eligible participants (mean age, 52 years; mean weight loss, 8.3 kg) provided a fecal sample that was processed into aFMT by frozen, opaque, and odorless capsules. The participants were then randomly assigned to groups that received 100 capsules containing their own fecal microbiota or placebo until month 14. The primary outcome was regain of the lost weight over the expected weight-regain phase (months 6–14). Secondary outcomes were gastrointestinal symptoms, waist circumference, glycemic status, and changes in the gut microbiome, as measured by metagenomic sequencing and 16s ribosomal RNA. We validated the results in a parallel in vivo

study of mice specifically fed with Mankai compared with control chow diet. **RESULTS:** Of the 90 participants in the aFMT trial, 96% ingested at least 80 of 100 oral aFMT or placebo frozen capsules during the transplantation period. No aFMT-related adverse events or symptoms were observed. For the primary outcome, although no significant differences in weight regain were observed among the participants in the different lifestyle interventions during months 6–14 (aFMT, 30.4% vs placebo, 40.6%; $P = .28$), aFMT significantly attenuated weight regain in the green-Mediterranean group (aFMT, 17.1%, vs placebo, 50%; $P = .02$), but not in the dietary guidelines ($P = .57$) or Mediterranean diet ($P = .64$) groups (P for the interaction $= .03$). Accordingly, aFMT attenuated waist circumference gain (aFMT, 1.89 cm vs placebo, 5.05 cm; $P = .01$) and insulin rebound (aFMT, −1.46 ± 3.6 μIU/mL vs placebo, 1.64 ± 4.7 μIU/mL; $P = .04$) in the green-Mediterranean group but not in the dietary guidelines or Mediterranean diet (P for the interaction $= .04$ and .03, respectively). The green-Mediterranean diet was the only intervention to induce a significant change in microbiome composition during the weight-loss phase, and to prompt preservation of weight-loss–associated specific bacteria and microbial metabolic pathways (mainly microbial sugar transport) after the aFMT. In mice, Mankai-modulated aFMT in the weight-loss phase compared with control diet aFMT, significantly prevented weight regain and resulted in better glucose tolerance during a high-fat diet–induced regain phase (all, $P < .05$). **CONCLUSIONS:** Autologous FMT, collected during the weight-loss phase and administrated in the regain phase, might preserve weight loss and glycemic control, and is associated with specific microbiome signatures. A high-polyphenols, green plant-based or Mankai diet better optimizes the microbiome for an aFMT procedure. ClinicalTrials.gov number, NCT03020186.

Keywords: Autologous FMT; Obesity; Weight Regain After Diet; Diabetes.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

This study evaluated the efficacy and safety of diet-modulated autologous fecal microbiota transplantation (aFMT) in preventing weight regain in 90 obese participants over 14 months and mice fed a Mankai diet.

NEW FINDINGS

aFMT, using fecal samples collected during a period of diet and weight loss, can prevent weight regain after the diet, and also increase glycemic control, possibly via specific microbiome signatures. This procedure is optimized by green, plant-based, weight loss diet.

LIMITATIONS

Most of the trial's participants were men.

IMPACT

Diet induced weight-loss can be preserved, along with glycemic control, for months after a diet via aFMT capsules. A green, plant-based diet can optimize the fecal microbiota for this procedure.

Weight regain and rebound of cardiometabolic risk factors after initial rapid weight loss have long been major challenges of durable dieting,[1] a phenomenon that was also observed in our and other previous long-term weight-loss trials.[2–4] Several mechanisms might explain the regain in body weight and cardiometabolic risk,[5,6] with the gut microbiota potentially serving as a modifiable treatment target.[7]

Fecal microbiota transplantation (FMT), that is, reconstitution of the gut microbiota by transplantation of stool from a healthy individual, offers a potent therapeutic approach in diseases mediated by gut dysbiosis, and is now considered standard of care in treatment of recurrent *Clostridioides difficile* infection.[8–10] As we reported previously, oral, capsulized, FMT as a route of administration can offer a safe FMT procedure in the outpatient setting.[11] The gut microbiota has repeatedly been associated with metabolic functions, and FMT from lean individuals mitigated obesity and detrimental metabolic traits in several animal experiments.[7,12–17] Preliminary studies suggest that transfer of a "lean microbiome" by FMT might modulate glycemic control without inducing weight loss in obese individuals.[9,10,18] However, human studies are sparse, possibly due to safety concerns and practical barriers associated with FMT.[19,20] Autologous FMT (aFMT) can serve as a viable alternative, as it was recently found to improve post-antibiotic microbiome reconstitution in 6 individuals.[21]

Increased consumption of plants, along with reduced consumption of red and processed meat, were linked to lower risk of obesity, type 2 diabetes, and all-cause mortality.[22–24] These favorable effects of plant-based diets were previously attributed to their increased fraction of plant polyphenols and dietary fibers,[25,26] with both components shown to have a prebiotic effect.[27]

The Dietary Intervention Randomized Controlled Trial Polyphenols-Unprocessed (DIRECT PLUS), aimed to examine whether the potential efficacy and safety of diet-modulated aFMT on weight regain attenuation is differently affected by distinct weight-loss interventions. We hypothesized that green-Mediterranean/high-polyphenols diet, enriched with green-tea and *Wolffia globosa* (Mankai) green plant, could be potent in optimizing the microbiome as the platform of successful subsequent aFMT.

Methods

Eligibility and Recruitment

The DIRECT PLUS (ClinicalTrials.gov Identifier: NCT03020186) weight-loss trial was a 2-phased randomized controlled trial involving overweight sedentary adults. This sub-study was conducted between May 2017 and July 2018

Abbreviations used in this paper: **aFMT, autologous fecal microbiota transplantation; KEGG, Kyoto Encyclopedia of Genes and Genomes; MET, metabolic equivalent for task.**

Most current article

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among employees of the nuclear research center in Dimona, Israel, an isolated facility with an on-site clinic and monitored provided lunch. Eligibility included age older than 30 years, abdominal obesity (waist circumference: men >102 cm, women >88 cm) or dyslipidemia (triglyceride >150 mg/dL and high-density lipoprotein cholesterol ≤40 mg/dL for men; ≤50 mg/dL for women). Exclusion criteria are provided in the Supplementary Material. The study was approved and monitored by the Soroka University Medical Center human subject committee. All participants provided written informed consent and received no financial compensation or gifts.

Randomization and Study Design

The trial included 2 phases: a randomized, open-label, lifestyle intervention and a randomized, double-blind, aFMT augmentation. In the first phase, participants were randomly assigned in a 1:1:1 ratio to 1 of 3 lifestyle intervention groups: physical activity and healthy dietary guidelines; physical activity and Mediterranean diet; or physical activity and green-Mediterranean diet. After 6 months of lifestyle intervention, the expected weight nadir based on our previous dietary interventional trials,[2,3] participants who lost at least 3.5% body weight were recruited to the double-blind, placebo-controlled, aFMT intervention as an augmentation to their assigned lifestyle intervention. The 3.5% cutoff was chosen by an estimation aimed to maximize statistical power, accounting for both expected sample size and weight difference, based on our previous CENTRAL trial (Supplementary Material).[2] Participants prescribed antibiotic therapy 2 months before randomization were excluded. Eligible participants were asked to deliver a full fecal sample that was processed into aFMT capsules. The participants were simultaneously randomized in a 1:1 ratio within sex and lifestyle intervention strata (Supplementary Material) to receive 10 g of aFMT (consumed as ten 1-g capsules) or identical placebo capsules delivered 10 times starting at 8 months after initiation of lifestyle intervention (2 months after fecal collection) during a 6-month period. A total of approximately 100 g fecal matter was consumed between months 8 and 14.

The initial protocol timed the administration period to months 10–12. However, in an attempt to robustly, and more persistently, counteract weight regain, the protocol was amended before intervention initiation to a 6-month administration period (months 8–14).

Lifestyle Intervention

All groups received free gym memberships and instructions to engage in moderate-intensity physical activity, approximately 80% of which was aerobic. The workout program included 45–60 minutes of aerobic training 3–4 times per week and 2–3 sessions of resistance training per week.

Healthy Dietary Guidelines

In addition to the workout program, participants received standard nutritional counseling to promote a healthy diet and to achieve a similar intervention intensity (defined as the intensity of group and personal guidance during the trial) to that of the other 2 arms.

Mediterranean Diet

In addition to the workout program, participants were instructed to adopt a calorie-restricted Mediterranean diet as described in our previous trials,[2,3] supplemented with 28 g/d of walnuts (containing 440 mg polyphenols) that was provided by the study team.

Green-Mediterranean Diet

In addition to the Mediterranean intervention (including the provided walnuts), the green-Mediterranean diet was designed to contain less red and processed meat compared with the Mediterranean diet and be richer in plants and polyphenols. Participants were provided with the following items: 4 cups/d green tea and 100 g frozen cubes of *Wolffia globosa* duckweed (Mankai cultivated strain) aquatic plant[28,29] consumed as a 500-mL green shake. The green-Mediterranean diet contained an additional 800 mg/d of polyphenols beyond that provided in the Mediterranean diet. Both Mediterranean and green-Mediterranean diets were equally calorie restricted (isocaloric), containing 1500–1800 kcal for men and 1200–1400 kcal for women.

All lifestyle interventions included 90-minute nutritional and physical activity sessions in the workplace. Sessions were weekly during the first month, once a month during the subsequent 6 months, and every other month thereafter (lifestyle interventions are detailed in Supplementary Material and Supplementary Table 1). Adherence to the diet was assessed by monitoring attendance in the sessions and quantified by a self-administered validated electronic 127-item food-frequency questionnaire.[30] Adherence to the exercise intervention was monitored during the group meetings and quantified using an electronic self-reported validated physical activity questionnaire.[31] Physical activity intensity levels were subsequently measured using metabolic equivalent for task (MET) units per week; each unit is defined as the ratio of work metabolic rate to the standard resting metabolic rate and MET levels can range from 0.9 METs (sleeping) up to 18 METs (fast running).[32]

Autologous Fecal Microbiota Transplantation Capsule Processing and Administration

Full fecal samples were collected at 6 months at the study site, immediately frozen to −20°C for 1–3 days, and then transferred to −80°C pending processing at the Center for Microbiome Research at Shamir Medical Center. After randomization, samples from participants allocated to the aFMT group were processed to aFMT capsules, as described previously[11] and as reported in the Supplementary Material. Each batch was divided in 10 equal doses of 10 capsules, transferred to the study site, and stored at −80°C pending administration. aFMT and placebo frozen capsules were opaque and odorless and identical in appearance. Placebo capsules consisted of agarose in normal saline/glycerol (the same vehicle as in aFMT capsules). The participants and investigators were blinded to the treatment group allocation. As the capsules were host-specific, a strict identification protocol was applied during each administration session. Administration sessions were held weekly for the first month, and every 3 weeks thereafter, for a total of 10 sessions. Each individual administration was directly observed by an investigator.

Complementary Mice Model

We utilized an obese mouse model, comparable with the human aFMT trial. The model was achieved by a 4-week high-

fat diet feeding of Swiss-Webster mice. The obese mice subsequently underwent a 4-week weight-loss phase, induced by normal-chow feeding, with an added daily Mankai gavage equivalent in quantity to the daily intake in the human trial (0.2 g/kg/d). Controls were fed with the same normal-chow diet, with the sole difference being daily saline gavage replacing the Mankai. After the weight-loss phase, stool samples were collected and processed to aFMT inocula. Next, both Mankai and control groups losing at least 8% underwent a weight-regain phase, induced by a 4-week high-fat diet, with each mouse receiving biweekly aFMT from its post–weight-loss fecal sample. Mice body weight was measured along the study, and insulin sensitivity was measured following the weight regain by intraperitoneal glucose tolerance test.

Blood, Fecal, and Clinical Measurements

Participants were weighed without shoes to the nearest 0.1 kg at baseline and 6 and 14 months. Waist circumference was measured halfway between the last rib and the iliac crest to the nearest millimeter at baseline and 6 and 14 months. An online symptoms questionnaire, based on Common Terminology Criteria for Adverse Events, version 5.0, was used to assess possible adverse effects 24 hours after intake of capsules, after the first, fourth, sixth, and eighth administration session. Blood samples were obtained after a 12-hour fast at baseline and at 6 and 14 months, centrifuged, and stored at −80°C until assayed (Supplementary Material). Presence of type 2 diabetes mellitus was defined for participants with baseline fasting plasma glucose levels $\geq$126 mg/dL or hemoglobin A1c levels $\geq$6.5% or if regularly treated with oral antihyperglycemic medications or exogenous insulin. Prediabetes was defined as fasting plasma glucose levels between 100 and 125 mg/dL or hemoglobin A1c levels in the range of 5.7%–6.4%. Fecal samples were collected at baseline and 6 and 14 months at the study site, immediately frozen to −20°C for 1–3 days, then transferred to −80°C pending DNA extraction for shotgun metagenomic sequencing and 16s ribosomal RNA, when appropriate. To characterize the microbiome, fecal DNA was extracted, sequenced, and normalized with an average depth of 15.4 ± 2.6 million reads per sample (mean ± standard deviation). DNA sequences were aligned using an accelerated version of the Needleman-Wunsch algorithm to a curated database containing all representative genomes in RefSeq, version 86. Each input sequence was assigned the lowest common ancestor that was consistent across at least 80% of all reference sequences tied for best hit. The number of counts for each taxon was then normalized to the average genome length. Species accounting for $<1 \times 10^{-3}$ of all species-level markers were discarded. Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology groups were observed directly using alignment against a gene database derived from the strain database used. Fecal samples were further sequenced on a MiSeq platform after amplification of V3–V4 hypervariable region of the 16S ribosomal RNA gene using the primer set 341F/806R, and processed by the DADA2 pipeline (see Supplementary Material). Participants prescribed antibiotic therapy 2 months before the delivery of baseline fecal samples were excluded from all microbiome analyses. Fecal samples handling and sequencing for the mice microbiome analysis is described in Supplementary Material.

All blood biochemical assays were performed at the University of Leipzig, Germany. Fecal metagenomic sequencing was performed at CoreBiome (New Brighton, MN). 16s ribosomal RNA sequencing was performed at Fondazione Edmund Mach (Trentino, Italy). Laboratory personnel were blinded to the randomized lifestyle and aFMT interventions.

Statistical Analysis

The primary outcome was weight regain, defined as percent weight change between 6 and 14 months from the initial 6-month weight loss. Secondary outcomes were gastrointestinal symptoms, waist circumference rebound, glycemic control, and gut microbiome changes. All outcomes were assessed by aFMT treatment effect, and by the interaction with green-Mediterranean diet.

The intention-to-treat analyses included all 90 participants by imputing the missing follow-up data (n = 1) of the primary outcome using the multiple imputation technique (Supplementary Material). Continuous variables are presented as mean ± SD and categorical variables are presented as total count. Differences between time points are expressed as absolute values, unless otherwise specified. To detect differences between treatment groups, t tests were used for parametric variables. Nonparametric variables and data determined to be non-normal after log-transformation were analyzed using the Mann-Whitney test. A linear regression model with analysis of variance was applied to assess the interaction between the green-Mediterranean diet and aFMT, and to assess the interaction between green-Mediterranean-specific components at time 6 months and aFMT. Intake of each component (ie, Mankai and green tea) was defined as high or low compared with the overall median intake. Microbial composition similarity between time points in each group was compared using permutational multivariate analysis of variance of weighted UniFrac distances. To evaluate changes in specific bacteria and KEGG module relative abundance, weight-associated bacteria/pathways were identified by comparing relative abundance change between the baseline and the 6-month time point, discarding bacteria/pathways with mean relative abundance $<1 \times 10^{-3}$. Next, aFMT-affected bacteria/pathways were screened by identifying those who remained significantly changed after 14 months in the aFMT group, excluding bacteria/pathways that changed in the placebo group as well. To assess to what extent microbial features were preserved by aFMT within lifestyle intervention strata, the number of features observed in the microbiome analysis were compared with a permuted null model with 1000 iterations, shuffling sample labeling at each iteration. Comparisons were made using Wilcoxon rank-sum test, applying Benjamini-Hochberg false discovery rate corrections for multiple comparisons. All microbiome analyses were validated using centered-log ratio transformation, accounting for data compositionality. Differences were considered significant for $P < .05$. Statistical analyses were performed using R software, version 3.5.3. All authors had access to the study data and reviewed and approved the final manuscript.

Results

Enrollment, Baseline Characteristics, and Adherence

After 6 months of dietary intervention, 155 of 294 (52.7%) DIRECT PLUS participants met the inclusion

criteria of 6-month weight loss with no recent antibiotic therapy. Of these, 90 subjects who consented were randomly assigned to aFMT (n = 44) or placebo (n = 46) (Figure 1). No significant differences in weight or anthropometric or metabolic characteristics were observed at 6 months and in 0- to 6-month changes between the 90 subjects enrolled to the 65 who declined. Baseline and 6-month characteristics of the participants, across treatment and lifestyle intervention groups, are presented in Table 1 and Supplementary Table 2. At baseline, mean age was 52 years and mean body mass index was 31.3 kg/m^2. Of the study population, 12.2% had type 2 diabetes and 36.7% had prediabetes. Ninety-one percent of participants were men, representing the workplace profile.

After 6 months of lifestyle intervention, mean weight loss was −8.27 ± 5 kg. No significant differences were observed between the randomized treatment groups in anthropometric or laboratory measures at 6 months, in total or within lifestyle intervention group strata. As reported previously,[28] the green-Mediterranean group was distinguished by decreased intake of red meat and poultry and increased intake of fish, green tea, and *Wolffia globosa* (Mankai) compared with the Mediterranean group. At 6 months, both Mediterranean diets reported lower carbohydrate and higher protein intake than the dietary guidelines group (all, $P < .01$), with no significant difference in fat intake ($P = .10$). At the end of the trial, no significant differences in the macronutrient intake were observed between the aFMT and placebo groups, across lifestyle intervention strata. All 3 intervention groups similarly increased their physical activity level compared with baseline. By the end of the trial, no significant difference was observed in the reported physical activity between the aFMT and placebo group, within all 3 lifestyle interventions (Supplementary Tables 4 and 5). The overall treatment compliance rate, defined as intake of >80 capsules, was 95.6%.

Safety and Symptom Monitoring

No severe adverse events were reported during the study period. After the first administration session, more participants in the placebo group reported bloating and flatulence compared with the aFMT group ($P = .04$). During the remainder of the study, no significant differences between groups were observed for any of a variety of symptoms (Figure 2).

Dietary and Autologous Fecal Microbiota Transplantation Effects on Weight Regain, and the Interaction With Green-Mediterranean Diet

The green-Mediterranean and Mediterranean groups exhibited a similar 0 to 6-month weight loss (ie, baseline), with both showing significantly greater reductions than the dietary guidelines group (−8.9 ± 5.6 kg, −8.8 ± 4.7 kg, and −5.4 ± 2.7 kg, respectively; $P = .03$ for both Mediterranean groups compared with the dietary guidelines group). Across treatment groups, the aFMT and placebo groups experienced a similar 0 to 6-month weight loss, before the capsule administration (aFMT −8.3 ± 5.1 kg vs placebo −8.3 ± 4.8 kg; $P = .92$).

The primary outcome was weight regain, defined as percent weight change between 6 and 14 months from the initial 6-month weight loss. After the capsule administration phase, there was no significant difference in weight regain between the dietary groups (dietary guidelines 39.1% ± 50.3%, Mediterranean 36.1% ± 40.2%, and green-Mediterranean 33.6% ± 45.4%; $P = .92$). Overall, regain was 30.4% ± 44% (+2.2 kg) in the total aFMT groups vs 40.6% ± 43% (+2.6 kg) regain in the total placebo groups ($P = .28$).

Examining the interaction between diet and treatment, aFMT significantly attenuated weight regain in the green-Mediterranean diet group (aFMT 17.1% ± 42.8% [+1.6 kg] vs placebo 50% ± 42.9% [+3.6 kg]; $P = .02$), but not in the dietary guidelines and Mediterranean groups ($P = .57$ and $P = .64$, respectively; P of interaction with green-Mediterranean diet = .03) (Figure 3*A*).

In an exploratory analysis, evaluating the interaction between aFMT and the intake of green-Mediterranean-specific components, that is, Mankai and green tea, on weight regain, increased frequency of Mankai intake at 6 months was associated with lower subsequent weight regain in the aFMT group compared with placebo (P of interaction = .04). A similar, although marginally significant, pattern was found in participants with increased green tea intake at 6 months (P of interaction = .06).

Dietary and Autologous Fecal Microbiota Transplantation Effects on Waist Circumference and Glycemic Status and Interactions With Green-Mediterranean Diet

The secondary outcomes were rebound of waist circumference and glycemic control.

Waist circumference change between 6 and 14 months was not different across dietary groups (dietary guidelines 0.7 ± 4.4 cm, Mediterranean 2.4 ± 5.1 cm, and green-Mediterranean 3.5 ± 4.5 cm; $P = .16$) or between treatment groups (aFMT 2.2 ± 4.5 cm vs placebo 3 ± 5.1 cm; $P = .58$). However, a significant attenuation was observed by aFMT in the green-Mediterranean group (aFMT 1.89 ± 4.9 cm vs placebo 5.05 ± 3.6 cm; $P = .01$) and not in the dietary guidelines and Mediterranean groups ($P = .56$, $P = .34$, respectively; P of interaction = .04) (Figure 3*B*).

Similarly, no significant difference was observed in 6- to 14-month change in fasting insulin levels across dietary groups (dietary guidelines 0.5 ± 3.7 μIU/mL; Mediterranean 0.8 ± 4.1 μIU/mL, and green-Mediterranean 0.2 ± 4.5 μIU/mL; $P = .82$) or between treatment groups (aFMT 0.06 ± 3.6 μIU/mL vs placebo 0.9 ± 4.6 μIU/mL; $P = .71$). However, we observed a significant difference in the 6- to 14-month change in fasting insulin levels between the aFMT (−1.46 ± 3.6 μIU/mL) and placebo (+1.64 ± 4.7 μIU/mL) groups in the green-Mediterranean group (P between treatment groups = .04), and no effect was observed within the dietary guidelines and Mediterranean groups ($P = .21$ and $P = .47$; P of interaction = .03) (Figure 3*C*).

Table 1. Baseline Characteristics of the Study Population

Characteristic	All subjects		Healthy dietary guidelines		Mediterranean diet		Green Mediterranean diet	
	aFMT	Placebo	aFMT	Placebo	aFMT	Placebo	aFMT	Placebo
Subjects, n	44	46	8	8	17	18	19	20
Sex, male, n	42	40	7	8	16	15	19	17
Diabetes, n (%)	8 (18)	3 (7)	2 (25)	0 (0)	0 (0)	1 (6)	6 (2)	2 (10)
Prediabetes, n (%)	15 (34)	18 (39)	4 (50)	2 (25)	5 (25)	6 (33)	6 (32)	10 (50)
Age, *y*, mean (SD)	53.14 (9.97)	51.63 (11.65)	52.43 (7.55)	52.05 (12.17)	54.49 (9.88)	52.61 (10.75)	52.24 (11.20)	50.57 (12.71)
Baseline characteristics, mean (SD)								
Body mass index, kg/m^2	30.89 (3.45)	31.39 (4.09)	30.57 (3.81)	29.66 (2.11)	30.93 (3.77)	31.92 (5.28)	30.97 (3.19)	31.60 (3.40)
Waist circumference, *cm*	108.91 (7.44)	109.59 (10.55)	108.25 (7.63)	102.38 (5.37)	109.18 (7.44)	113.17 (13.72)	108.95 (7.76)	109.25 (7.12)
Weight, *kg*	93.74 (14.12)	92.20 (14.45)	90.41 (15.44)	83.22 (9.82)	93.12 (13.58)	96.24 (17.24)	95.70 (14.50)	92.16 (11.98)
Fasting plasma glucose, *mg/dL*	101.66 (19.22)	100.16 (15.75)	101.17 (16.19)	95.78 (13.96)	97.86 (7.02)	99.25 (11.04)	105.24 (26.52)	102.73 (19.77)
Serum triglycerides, *mg/dL*	144.30 (57.82)	131.56 (68.75)	149.68 (58.79)	191.38 (123.11)	147.00 (62.76)	129.39 (53.90)	144.30 (57.82)	131.56 (68.75)
Serum HDL cholesterol, *mg/dL*	44.61 (10.30)	47.78 (13.02)	41.94 (7.71)	49.37 (15.32)	42.65 (9.61)	42.37 (8.67)	44.61 (10.30)	47.78 (13.02)
6-month characteristics, mean (SD)								
Weight loss from baseline, *kg*	–8.28 (5.16)	–8.25 (4.85)	–6.20 (3.06)	–4.65 (2.26)	–8.23 (4.01)	–9.38 (5.27)	–9.21 (6.54)	–8.68 (4.71)
Body mass index, kg/m^2	28.17 (2.95)	28.60 (3.36)	28.47 (3.56)	28.03 (1.86)	28.19 (3.35)	28.84 (4.03)	28.02 (2.42)	28.62 (3.29)
Weight, *kg*	85.46 (12.30)	83.95 (11.74)	84.21 (14.62)	78.58 (8.25)	84.89 (12.54)	86.86 (12.87)	86.49 (11.67)	83.48 (11.52)
Waist circumference, *cm*	99.30 (7.32)	99.22 (8.02)	98.38 (8.25)	97.75 (6.80)	99.76 (8.50)	101.89 (6.94)	99.26 (6.06)	97.40 (9.02)
Fasting plasma glucose, *mg/dL*	97.12 (14.78)	94.71 (8.30)	93.39 (19.90)	92.17 (6.72)	94.63 (7.18)	94.17 (8.07)	101.14 (17.26)	96.22 (9.10)
Fasting plasma insulin, $\mu U/mL$	10.17 (5.08)	10.63 (5.12)	9.38 (5.54)	11.99 (5.36)	9.18 (3.54)	10.09 (3.50)	11.54 (6.06)	10.57 (6.28)
HOMA-IR	2.56 (1.63)	2.49 (1.22)	2.37 (1.97)	2.77 (1.33)	2.15 (0.85)	2.34 (0.82)	3.06 (1.98)	2.52 (1.50)

NOTE. No significant differences were observed between placebo or aFMT group in the measured baseline characteristics, overall, and across lifestyle interventions. HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

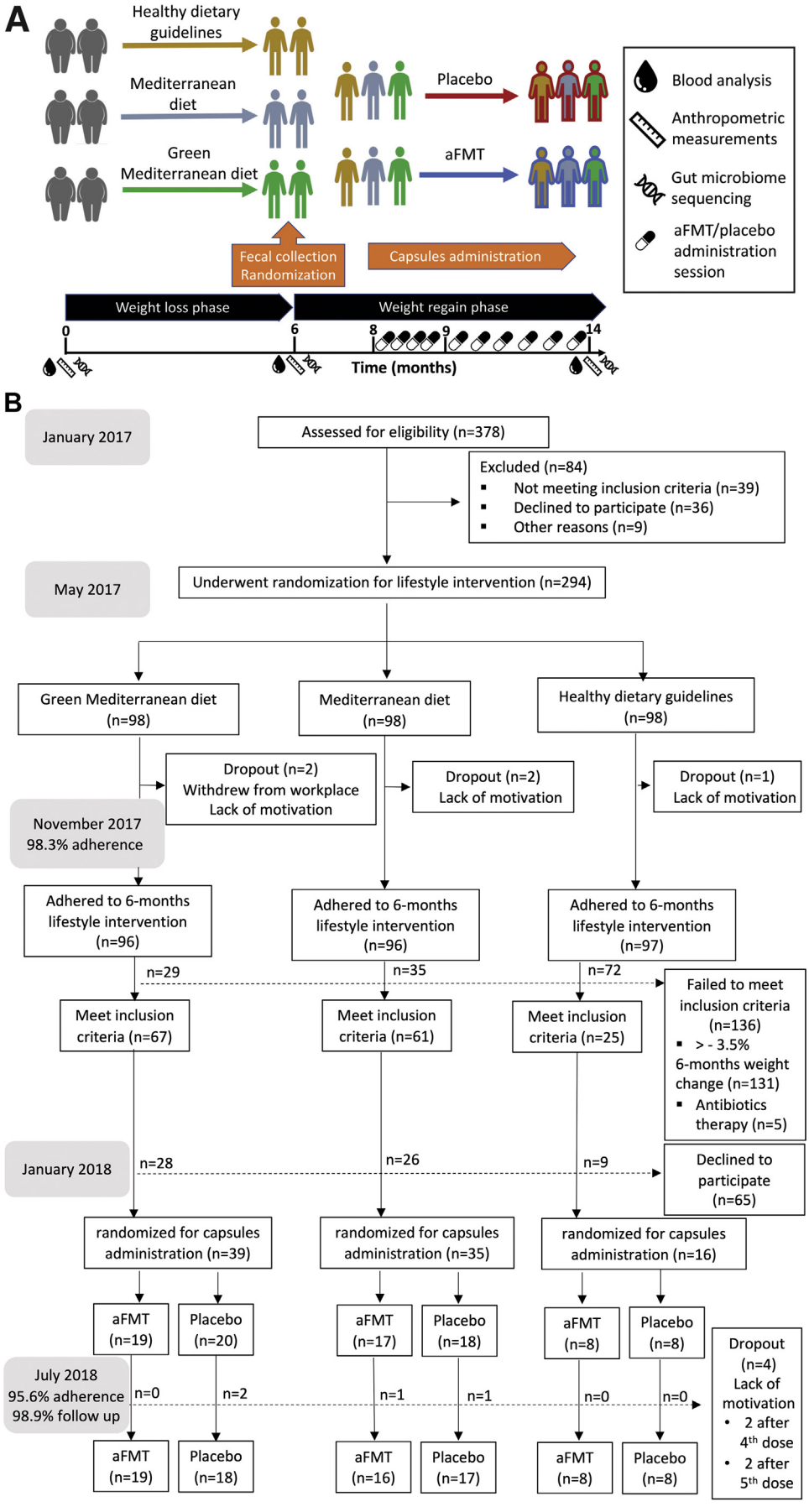

Figure 1. Study design, enrollment of the participants and completion of the study. (*A*) Experimental design. (*B*) Trial flow chart.

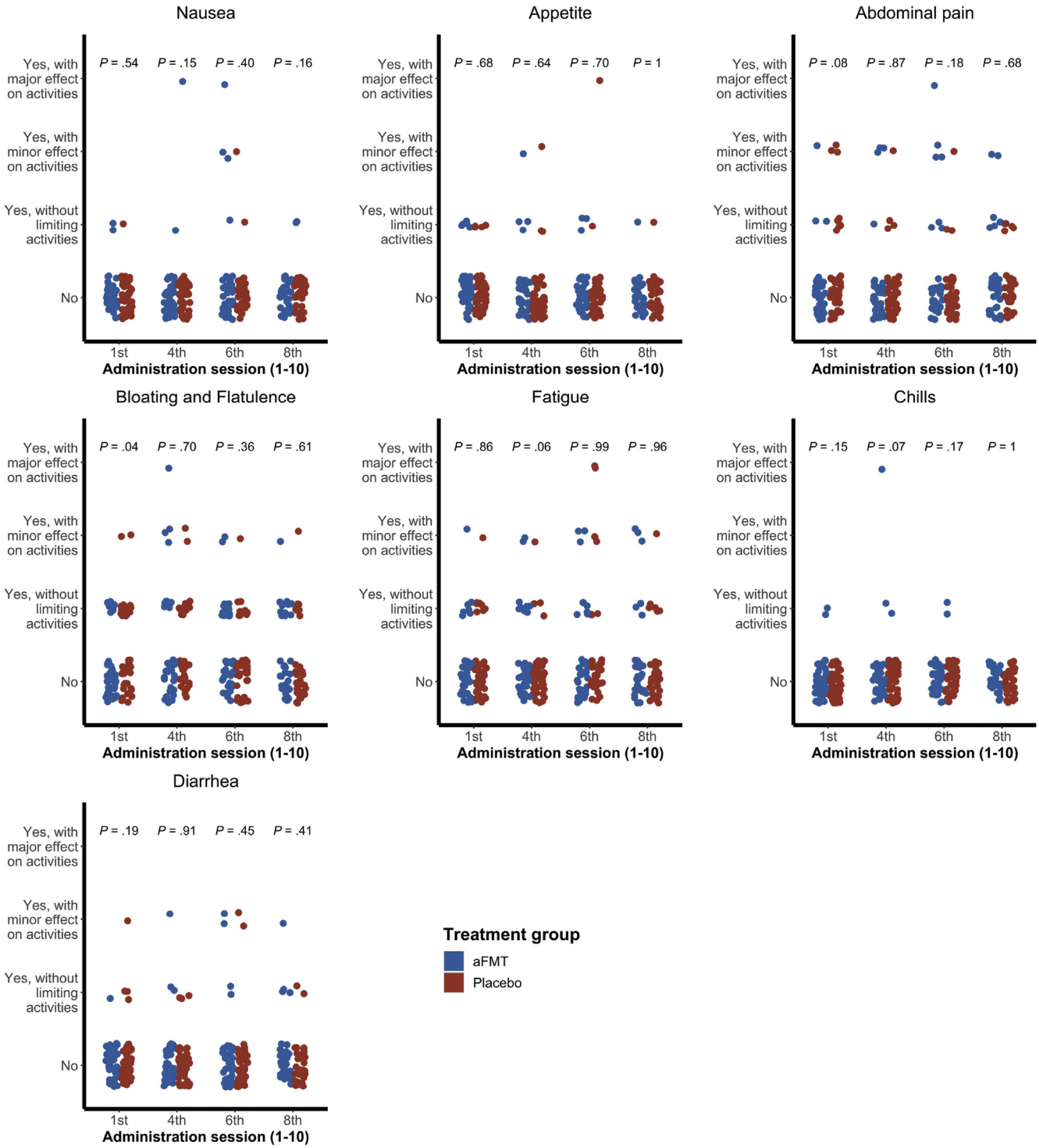

Figure 2. Symptom monitoring. Comparison between aFMT and placebo treatment groups in monitored symptoms. *P* values represent comparisons between treatment groups at each time point.

A similar pattern could be observed in Homeostatic Model Assessment of Insulin Resistance (*P* between dietary group = .72; *P* between treatment groups = .69; *P* aFMT vs placebo within the green-Mediterranean group = .08; *P* of interaction = .07) (Supplementary Figure 1). No differences were observed in 6- to 14-month fasting plasma glucose change across dietary groups ($P = .38$), treatment groups ($P = 1$), and the interaction between the 2 ($P = .97$).

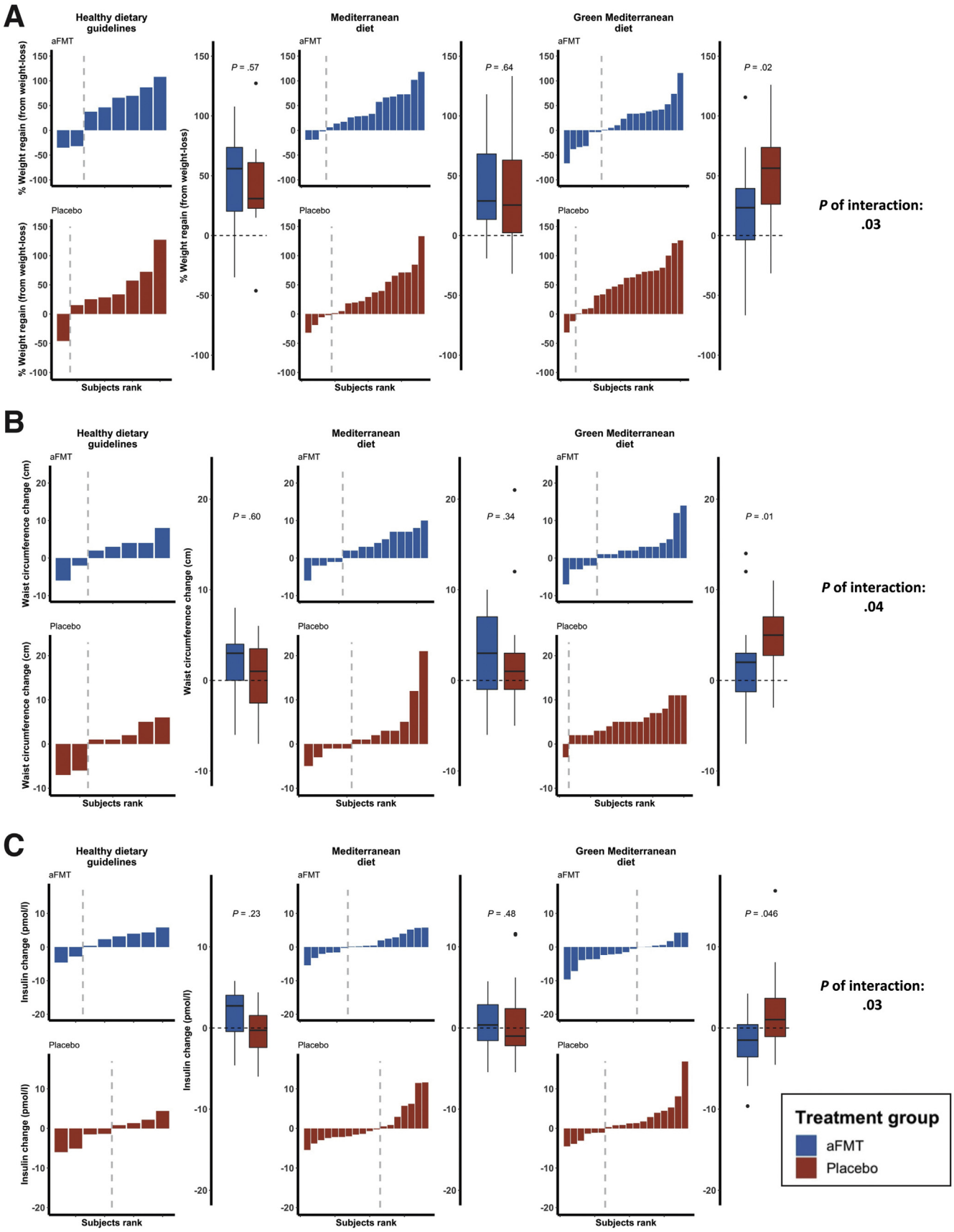

Figure 3. aFMT effect across lifestyle interventions; 6- 14-month changes in anthropometric measures and glycemic state. Body weight (*A*), waist circumference (*B*), and insulin (*C*) changes between 6 and 14 months. *Regain % was calculated as 100 × (14 months – 6 months) / (6 months – 0 months).

Microbiome Functional and Compositional Changes

Comparing microbiome composition change at the weight-loss phase (0–6 months) across lifestyle intervention groups, a significant shift could be observed between 0 and 6-month fecal samples in the green-Mediterranean group ($P = .004$), but not in the Mediterranean ($P = .25$) and dietary guidelines ($P = .63$) groups (Figure 4*A*). After the weight-regain phase (6–14 months) the aFMT green-Mediterranean group had the most stable gut microbiome composition, although no significant differences were present between 6- and 14-month samples within any of the subgroups (Figure 4*B*).

No species/metabolic pathways were observed to be affected by diet and aFMT in the dietary guidelines group.

During the weight-loss phase, among the Mediterranean group, 64 microbial metabolic pathways and 17 species were significantly changed. The most prominent changes were the increased abundance of *Akkermansia muciniphila* and 2 sulfate degradation pathways, along with the decrease in *Lactobacillus ruminis* and the oxidative phase of the pentose phosphate pathway. A single bacterial species, *Roseburia hominis*, was observed to retain the Mediterranean group's induced change by aFMT.

In the green-Mediterranean group, 47 microbial metabolic pathways and 18 species were significantly altered. The most prominent changes were the increase in abundance of *Bacteroides massiliensis* and *Paraprevotella clara,* along with the increase in LPS biosynthesis and type IV secretion system pathways. Of these, after the regain phase, 15 pathways and 6 species remained significantly changed among the green-Mediterranean participants in the aFMT group alone, not retaining the weight-loss–induced changes in the placebo group. Among the green-Mediterranean group, the species *Alistipes putredinis* and *Bacteroides vulgatus* increased in abundance and several microbial sugar transport pathways were down-regulated during the weight-loss phase, preserving the changes only in the aFMT group (Figures 4*C* and 5*A*, Supplementary Figures 2, 3, 5, and 6). The number of pathways and bacteria preserving the weight-loss–induced changes by aFMT compared with the expected number by a permuted null model, was significantly higher in the green-Mediterranean group (taxa: $P = .002$; KEGG modules: $P = .03$), but not in the dietary guidelines group (taxa: $P = 1$; KEGG modules: $P = 1$) and the Mediterranean group (taxa: $P = .08$; KEGG modules: $P = 1$). As several sugar-related pathways were among the preserved KEGG pathways, we evaluated whether the 6- to 14-month changes in these pathways were associated with insulin rebound within the aFMT green-Mediterranean group. Insulin rebound was found to be significantly associated with 13 of 15 preserved pathways (Figure 5*B*).

Mankai-Modulated Autologous Fecal Microbiota Transplantation Effect on Weight Regain and Glucose in Mice

During the weight-loss phase, both mice groups had a similar weight-loss pattern (n = 10; Mankai-fed –5.92 g vs control –5.48 g; $P = .75$) (Figure 6*B*). However, in the microbiome administration phase, the Mankai-aFMT group attenuated the high-fat diet–induced weight regain compared with control mice (Mankai 5.32 g vs control 8.88 g; $P = .03$) (Figure 6*C* and *D*). Comparing glucose tolerance of both aFMT groups after the regain phase, the Mankai-aFMT group had significantly lower glucose levels at 15 minutes and 30 minutes in a 120-minute intraperitoneal glucose tolerance test (Figure 6*E*). Assessing the mice gut microbiome composition change during the weight-regain phase, both groups had undergone a prominent composition shift after the administration of high-fat diet. However, the Mankai-aFMT group tended to preserve the weight-nadir composition compared with the control (week 11, $P = .016$; week 12, $P = .056$; Figure 6*F*).

Discussion

In this 14-month human trial including 90 participants, green-Mediterranean/high-polyphenols diet was the only lifestyle strategy that induced a significant change of the gut microbiome composition during the weight-loss phase, potentially optimizing the conditions for aFMT derived from the maximal weight-loss phase. Human aFMT appeared to be a safe procedure during 6 months of administration. The observed compositional change in the gut microbiome of the green-Mediterranean group was subsequently associated with attenuated weight regain, waist circumference change, and reduced insulin rebound, after repeated administration in the form of aFMT. The aFMT's beneficial effect in the green-Mediterranean group coincided with the stabilization of weight-loss–associated changes in specific bacteria and microbial metabolic pathways, mainly related to sugar transport. In a mouse model, we were able to reproduce the effects of weight-nadir based aFMT on weight regain and insulin sensitivity, and to isolate the specific contribution of Mankai supplementation (one of the main components in the human intervention) to induce these effects.

The trial has several limitations. The study was conducted in a unique workplace with a vast majority of men. Combining 3 lifestyle strategies with aFMT treatment provided an opportunity to explore interactions between dietary strategies and aFMT intervention, but reduced the sample size in each lifestyle group. Furthermore, due to the nature of the lifestyle interventions and eligibility criteria of the aFMT study, the sample size of the dietary guidelines group was lower than those from the 2 Mediterranean diets. For ethical and technical reasons, the gut microbiome was assessed in fecal matter and not in bioactive sites along the gastrointestinal tract. Moreover, by administering minimally processed aFMT, specific bacteria that might have been more potent in this intervention were not isolated. Major strengths of this study include the simultaneous scheme in which all of the trial phases were performed at the same time, the double-blinded placebo-controlled design, the strictly monitored capsule administrations, the high adherence rate and the relatively large sample size, the duration of intervention and follow-up, and the validation of the results in a parallel mice model.

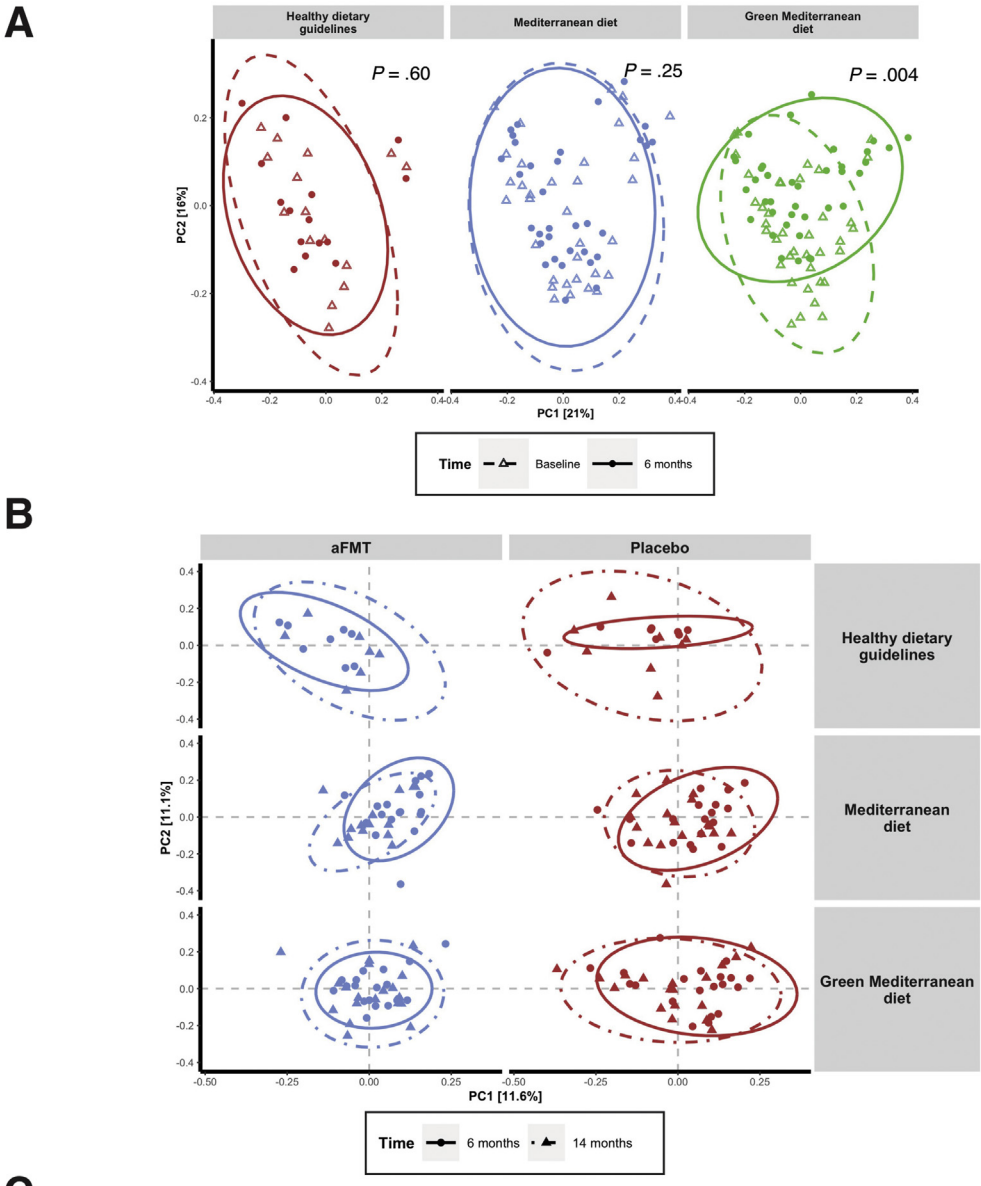

Figure 4. Microbiome composition and species-level changes. (*A*) Principal coordinate analysis (PCoA) of weighted UniFrac distances of microbiome composition measured by 16s ribosomal RNA sequencing. Each *subplot* displays the distances between individual microbiome samples within the distances of the indicated lifestyle intervention, at baseline and 6 months. (*B*) PCoA of Bray-Curtis distances of microbiome composition, each *subplot* displays the distances between individual microbiome samples within the indicated lifestyle intervention and treatment group, at 6 and 14 months. The 95% standard error ellipses are shown for each sub-group. (*C*) Shotgun metagenomics assessed species (*B*) that were significantly changed by weight loss and maintained by the aFMT treatment across lifestyle intervention groups.

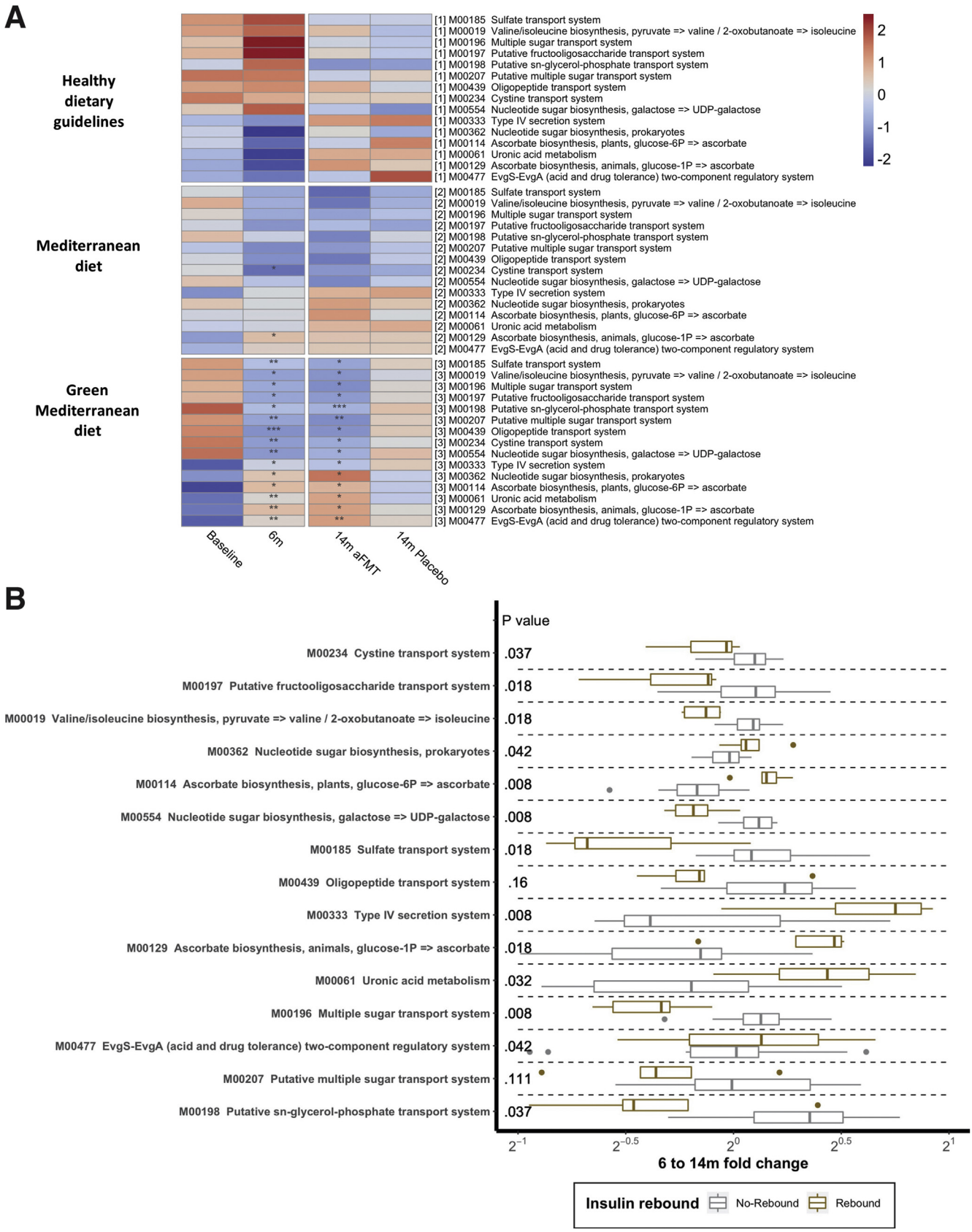

Figure 5. KEGG pathway changes and association with insulin rebound. (*A*) Shotgun metagenomics assessed metabolic pathways (KEGG modules) that were significantly changed by weight loss and maintained by the aFMT treatment, across lifestyle intervention groups. (*B*) Fold-change of the same metabolic pathways as in (*A*), comparing individuals with and without insulin rebound (defined as the median change between 6 and 14 months) among the aFMT + green-Mediterranean group participants.

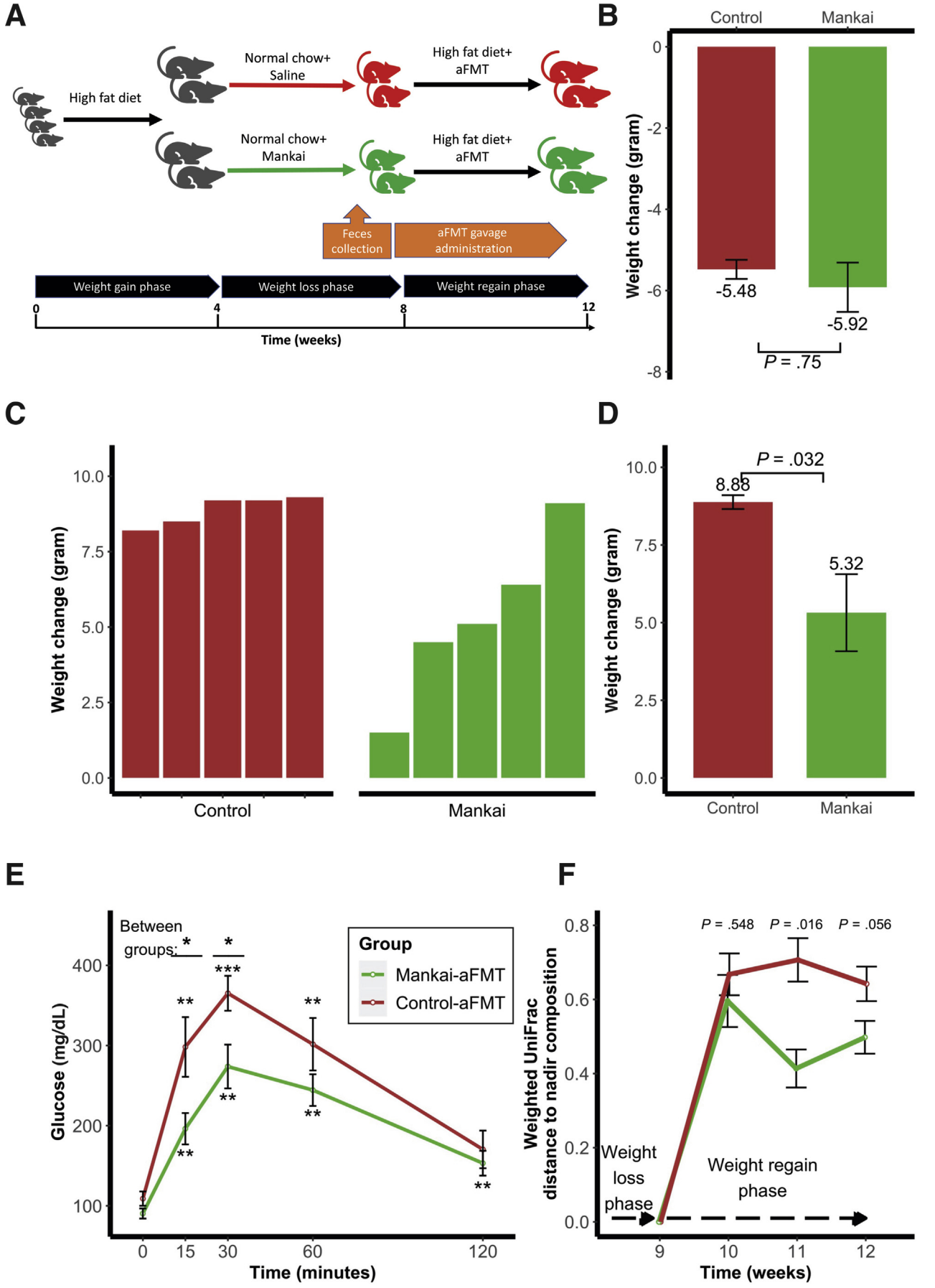

Figure 6. Mankai supplementation during weight loss and aFMT efficacy in mice experimental design (*A*); Mankai enrichment during weight loss and weight change after the weight-loss period (*B*); weight regain by high-fat diet + aFMT after weight loss with added Mankai/saline gavage (*C, D*) Intraperitoneal glucose tolerance test (IPGTT) after the weight regain phase (week 12) (*E*). Weighted UniFrac distances to the weight-nadir time point during the weight regain phase (*F*). *$P \leq .05$; **$P \leq .01$; ***$P \leq .001$.

No significant adverse events or gastrointestinal symptoms were found to be related to the aFMT treatment. These safety data are critical, as FMT has garnered attention as a potential therapeutic option to promote weight loss.[9,10,12,20] Despite rigorous donor screening, safety concerns remain when using donor-derived biotherapeutics. As aFMT eliminates potential transmission of pathogens, it is likely to be a safe alternative.

The potential of FMT in human adiposity has already been established,[12] using FMT from twin pairs discordant for obesity into germ-free mice. The findings suggested that the lean/obese phenotype was at least partially transmissible, and that the "lean" microbiome dominates the gut and attenuates the "obese" microbiome's effects on adiposity.[12] Consistent with the current study, randomized human studies that evaluated the effect of lean-to-obese allogenic FMT ($n = 18$[9], $n = 36$[10]) showed a beneficial response on insulin sensitivity.

We observed a sustained increase in specific microbial taxa in the green-Mediterranean group by the aFMT treatment, including the species *A putredinis, B vulgatus*, and *Bacteroides uniformis.* Notably, all 3 were previously associated with a lean state.[12,33–35] Interestingly enough, *A putredinis and B vulgatus* were associated not only with reduced body weight in the host, but with successful invasion from a lean host to an obese host.[12] It is plausible that the metabolic effect of aFMT in the green-Mediterranean group was partly mediated through the persistent colonization of these bacteria by aFMT during the regain phase.

In addition, we observed a sustained change in several microbial metabolic pathways that were reported to be associated with body weight, including "valine, leucine and isoleucine degradation," "ascorbate biosynthesis, [animals; glucose−1P = > ascorbate]" and "putative sugar transport system."[33] In an analysis aimed to evaluate the link between these pathways and insulin rebound, the first 2 were found to be associated, possibly affecting host insulin sensitivity.

Based on these results, the green-Mediterranean diet likely influenced the host environment, altering the gut microbiome composition and facilitating metabolic memory retention as represented by the attenuation of body weight and insulin rebound. Polyphenols are known for their prebiotic effect,[27] which might underlie the polyphenols' contribution to the prevention of weight regain, as shown in mice previously.[7] Notably, although no significant difference in weight regain was observed between the placebo groups across dietary interventions, the placebo-green-Mediterranean group regained the greatest amount of weight.

This trial brings to light a novel approach to weight-loss maintenance by microbiome optimization and conservation. However, this strategy should be investigated further as to which specific dietary components could modify the host microbiome potency.

In conclusion, the results suggest that beyond weight loss, dietary composition modifies the microbiota, and consequently, the therapeutic effect of aFMT by a combined "prebiotics to probiotics" model, induced by specific microbiome-modulating diet, and administrated as aFMT.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dxdoi.org/10.1053/j.gastro.2020.08.041.

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Conflicts of interest

These authors disclose the following: Iris Shai advises the nutritional committee of Hinoman, Ltd. Ilan Youngster is an advisor for Mybiotics Ltd. Dan Knights is CEO of, and holds equity in, CoreBiome, Inc. The remaining authors disclose no conflicts.

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Supplementary Methods

Inclusion and Exclusion Criteria

Inclusion criteria were age older than 30 years; waist circumference >102 cm in men and >88 cm in women; or dyslipidemia (triglyceride >150 mg/dL and high-density lipoprotein cholesterol ≤40 mg/dL for men and ≤50 mg/dL for women). Exclusion criteria were inability to perform physical activity; serum creatinine ≥2 mg/dL; serum alanine aminotransferase or aspartate aminotransferase more than 3 times above upper limit of normal; major illness that might require hospitalization; pregnancy or lactation; active cancer, or chemotherapy treatment in the last 3 years; warfarin treatment; pacemaker; and participation in a different trial.

Sample Size Calculation

Minimal required sample size was estimated based on the weight-loss pattern in our previous CENTRAL trial.[2] Aiming to detect a complete attenuation of the expected 3 ± 4.3 kg weight regain, for an α of 5% and power of 90%, the calculated sample size was 88 subjects in total.

Randomization Protocol and Allocation Sequence

Recruitment to the DIRECT PLUS and aFMT trial was performed by Ehud Rinott, Anat Yaskolka Meir, Hila Zelicha, Alon Kaplan, and Gal Tsaban. All eligible participants who signed consent to participate in the trial and completed the baseline measurements were randomized into 1 of the 3 intervention groups (healthy dietary guidance, Mediterranean, green-Mediterranean) at a 1:1:1 ratio and within strata of sex and work status (to ensure equal workplace-related lifestyle features between groups).

Participants reducing >3.5% body weight, without prescribed antibiotics 2 months before feces sampling who agreed to participate were randomized in 1:1 ratio to 2 treatment groups (aFMT and placebo) within strata of sex and lifestyle intervention group.

Randomization was conducted in a single phase using an ad-hoc R-based procedure, the randomization was done by Nirit Keren from the Center for Microbiome Research at Shamir Medical Center, Israel.

Lifestyle Intervention

All trial participants received free gym memberships and educational sessions, and were encouraged to engage in moderate intensity physical activity, approximately 80% of which included an aerobic component. The aerobic effort increased gradually, starting with 20 minutes of aerobic training at 65% maximum heart rate, and increased to 45–60 minutes of aerobic training at 80% of maximum heart rate. The full workout program included 45–60 minutes of aerobic training 3–4 times/week, and resistance training started with one set of weights corresponding to 60% of the maximum weight, eventually reaching the use of 2 sets of weights corresponding to 80% of the maximum weight. The resistance training included leg extensions, leg curls, squats, lateral pull-downs, push-ups, shoulder presses, elbow flexions, triceps extensions, and bent leg sit-ups. Physical activity group participants received basic health-promoting guidelines for achieving a healthy diet.

Autologous Fecal Microbiota Transplantation and Placebo Capsules Processing

Capsules processing was carried out under aerobic conditions. A fecal suspension was generated in normal saline without preservatives using a commercial blender. Materials were sequentially sieved to remove particulate material. The final slurry was concentrated by centrifugation and resuspended in saline at 10% of the volume of the initial sample with 20% glycerol added as a bacterial cryoprotectant. Fecal matter suspension was pipetted into size 0 capsules (650 μL), which were closed and secondarily sealed in size 00 acid-resistant hypromellose capsules (DRCaps; Capsugel, Morristown, NJ). Each sample was processed separately, and a dose of 100 capsules containing sieved, concentrated material, was derived from the fecal matter. Placebo capsules consisted of agarose in normal saline/glycerol (the same vehicle as in aFMT capsules).

Blood Sample Analysis

Serum levels of total cholesterol (coefficient of variation = 1.3%), low-density-lipoprotein cholesterol were determined enzymatically with a Cobas 6000 automatic analyzer (Roche. Basel, Switzerland). Plasma insulin levels were measured with the use of an enzymatic immunometric assay (Immulite automated analyzer; Seimens, Munich, Germany; coefficient of variation = 2.5%).

Plasma levels of high-sensitivity C-reactive protein were measured by enzyme-linked immunosorbent assay (DiaMed; Cressier, Switzerland; coefficient of variation = 1.9%). Plasma leptin levels were assessed by enzyme-linked immunosorbent assay (Mediagnost, Reutlingen, Germany), with a coefficient of variation of 2.4%.

Metagenomics Analysis BoosterShot Pipeline

DNA extraction. Samples were extracted using MO Bio PowerFecal (Qiagen, Hilden, Germany) automated for high throughput on QiaCube, with bead beating in 0.1-mm glass bead plates.

DNA quantification. Samples were quantified with Qiant-iT Picogreen dsDNA Assay (Invitrogen, Carlsbad, CA).

Library preparation and sequencing. Libraries were prepared with a procedure adapted from the Nextera Library Prep kit (Illumina, San Diego, CA). Libraries were sequenced on an Illumina NextSeq using single-end 1 × 145 reads with a NextSeq 500/550 High Output v2 kit (Illumina).

Sequence quality control. DNA sequences were filtered to remove low quality (Q-score <20) reads, and for length (<50), and adapter sequences were trimmed using cutadapt. Fastq files were converted a single fasta using shi7.

Operational taxonomic unit picking. DNA sequences were aligned to a curated database containing all representative genomes in RefSeq for bacteria with additional manually curated strains. Alignments were made at 98% identity against all reference genomes. Each input sequence was compared to each reference sequence in CoreBiome's Venti database using full gapped alignment with Burst. Ties were broken by minimizing the overall number of unique gene hits. For taxonomy assignment, each input sequence was assigned the lowest common ancestor that was consistent across at least 80% of all reference sequences tied for best hit. The number of counts for each taxon was then normalized to the average genome length. Species accounting for $<1 \times 10^{-6}$ of all species-level markers were discarded. Samples with <1000 sequences were also discarded. The normalized and filtered tables were used for all downstream analyses.

Functional genome content. Functional groups were observed directly using KEGG orthology groups by alignment against a gene database derived from the strain database used above.

16s Ribosomal RNA Sequencing Pipeline

DNA extraction. Fecal microbiota DNA was extracted using QIAamp PowerFecal DNA Kit (Qiagen) and a FastPrep-24 bead beater (MP Biomedicals, Santa Ana, CA). DNA quantity and quality was assessed spectrophotometrically by NanoDrop (ThermoFisher Scientific, Waltham, MA).

Library Preparation and Sequencing

For 16S ribosomal RNA sequencing amplification of total genomic fecal DNA was carried out using the specific bacterial primer set 341F (5′ CCTACGGGNGGCWGCAG 3′) and 806R (5′ GACTACNVGGGTWTCTAATCC 3′) with overhang Illumina adapters, targeting a approximately 460-bp fragment of the 16S ribosomal RNA variable region V3–V4.[2,3]

Polymerase chain reaction (PCR) amplification of each sample was carried out using 25-μL reactions with 0.2 μM of each primer and 12.5 ng template DNA, and employing KAPA HiFi HotStart ReadyMix. PCR amplification was carried out using a GeneAmp PCR System 9700 (ThermoFisher Scientific) with the following steps: 2 cycle at 94°C for 5 minutes, 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and 1 final elongation step at 72°C for 5 minutes. The PCR products were checked on 1.5% agarose gel and cleaned from free primers and primer dimer using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA) following the manufacturer's instructions. Subsequently dual indices and Illumina sequencing adapters Nextera XT Index Primer (Illumina) were attached by 7 cycles PCR (16S Metagenomic Sequencing Library Preparation, Illumina).

The final libraries were quantified using the Quant-IT PicoGreen dsDNA assay kit (ThermoFisher Scientific) by the Synergy2 microplate reader (Biotek, Winooski, VT), then libraries were pooled in an equimolar way and analyzed on a Typestation 2200 platform (Agilent Technologies, Santa Clara, CA). Barcoded library were sequenced on Illumina MiSeq (PE300) platform (MiSeq Control Software, version 2.0.5 and Real-Time Analysis software, version 1.16.18). Sequences with expected error rate $>1.5\%$ were removed from analysis.

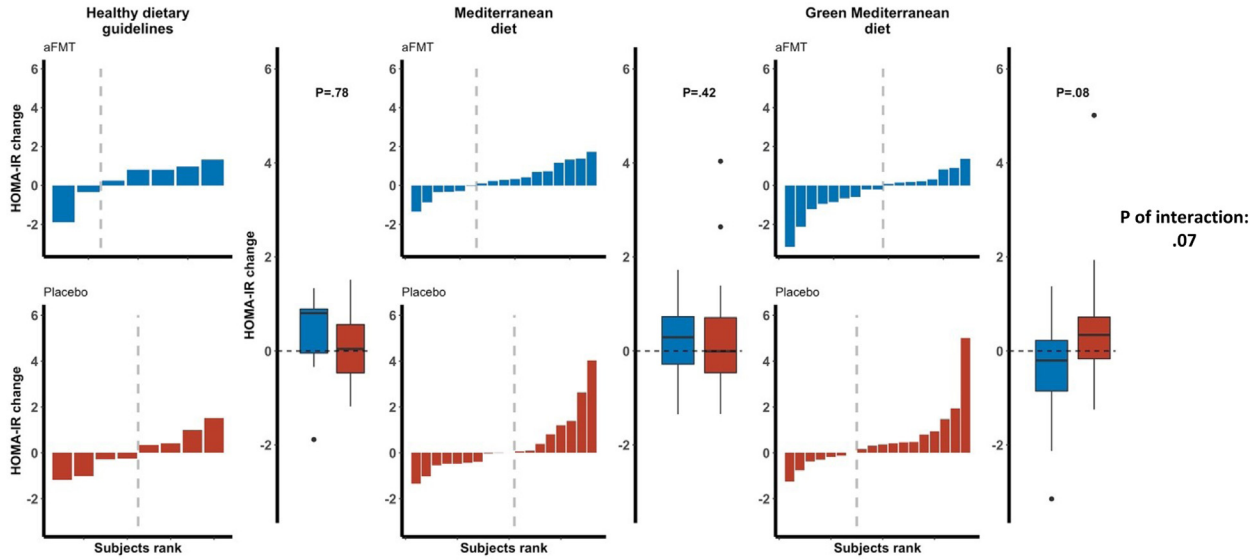
Mice Study Extraction, Amplification, and Sequencing of Fecal Samples

DNA was extracted from all fecal samples by PureLink Microbiome DNA Purification Kit (Invitrogen). The V4 region of the bacterial 16S ribosomal RNA gene was amplified by PCR using the 515F (AATGATACGGCGACCACCGAGATCTACACGCT) barcoded and 806R (TATGGTAATTGTGTGYCAGCMGCCGCGGTAA) primers. A reaction containing a final concentration of 0.04% of each primer and 0.5% of PrimeSTAR Max DNA Polymerase (Takara, Mountain View, CA) 50 μL total volume. PCR reactions were carried out by 35 cycles of denaturation (95°C), annealing (55°C) and extension (72°C), with final elongation at 72°C. PCR products were purified using AMPure magnetic beads (Beckman Coulter, Indianapolis, IN) and quantified using double-stranded DNA fluorescence quantification assay kit (DeNovix Inc, Wilmington, DE). Samples were then pooled at equal amounts of 50 ng, loaded on 2% agarose E-Gel (ThermoFisher), purified and sent for sequencing using the Illumina MiSeq platform (Genomic Center, Azrieli Faculty of Medicine, BIU, Israel).

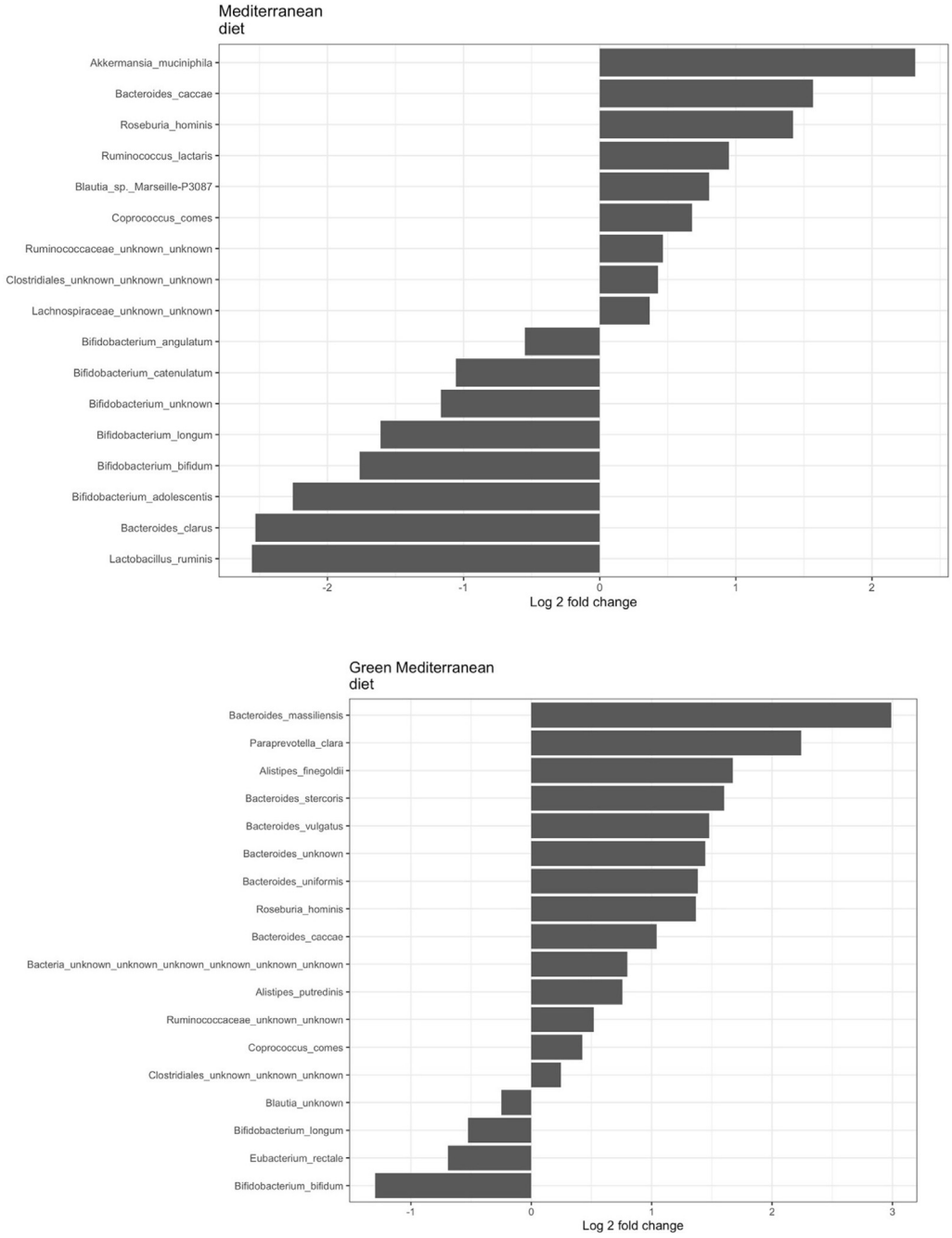
Multiple Imputations for Missing Follow-Up Data

We performed intention-to-treat analyses, including all 90 participants, by imputing missing follow-up data of a single participant on the 14-month time point of both primary outcomes by the multiple imputation technique.[4]

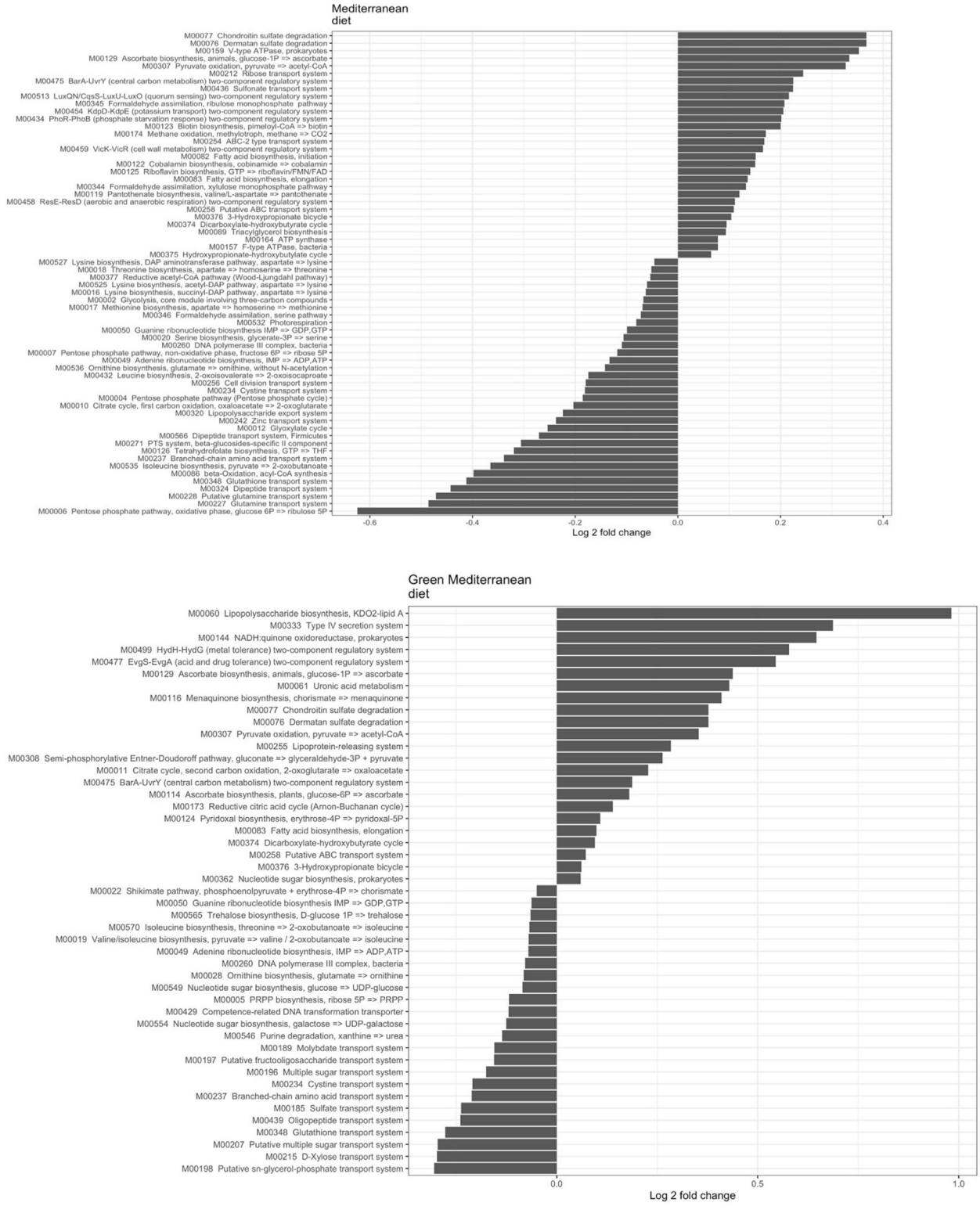
The imputation was done by the R package "mice,"[5] wherein the following predictors were used in the imputation model: age, sex, baseline weight, and initial 6-month weight loss.



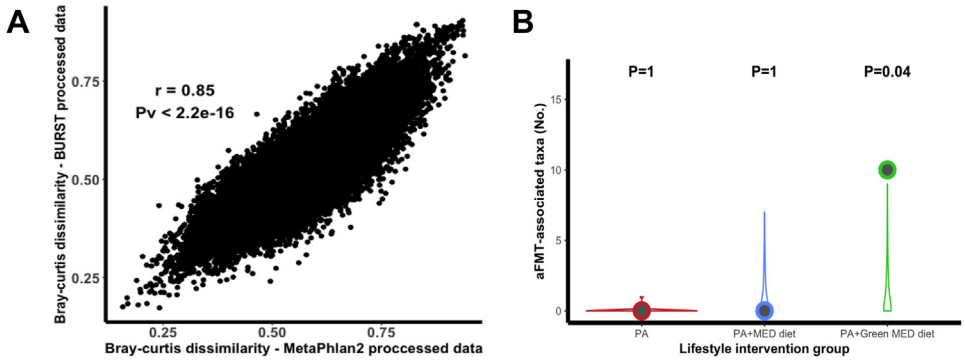
Supplementary Figure 1. aFMT effect on 6- to 14-month changes in Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) across lifestyle intervention.



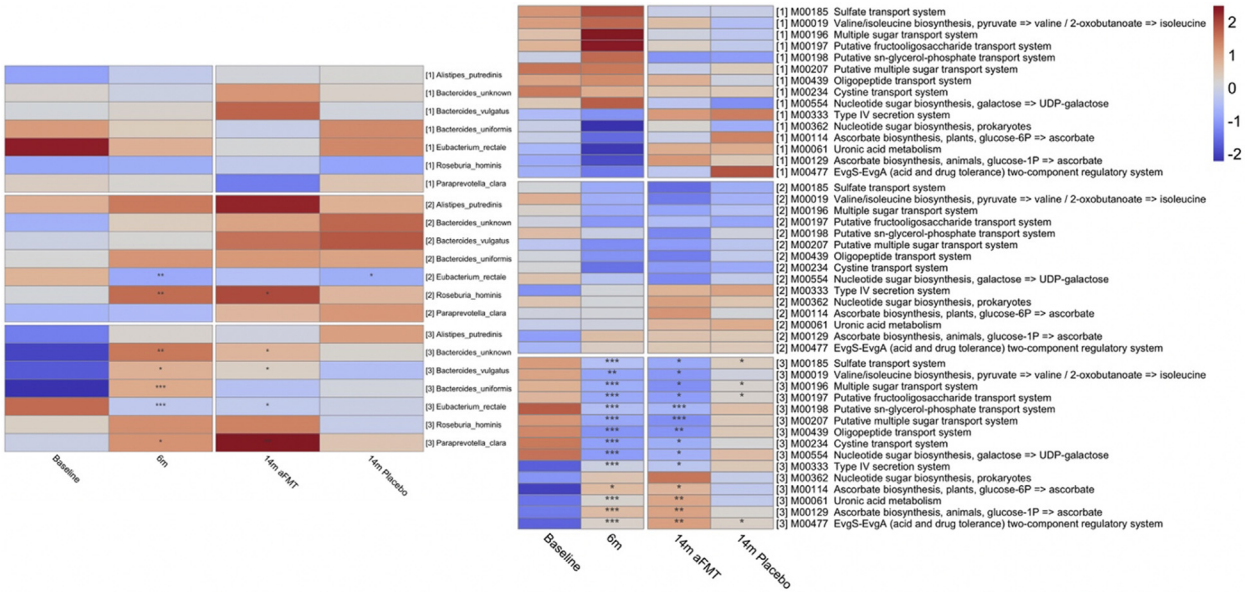
Supplementary Figure 2. 0 to 6-month taxa changes across lifestyle intervention arms. Species that were significantly changed by weight loss, across lifestyle intervention groups. Values represents log2 fold-change from baseline, by bacteria. No significant changes were observed among the healthy dietary guidance group.



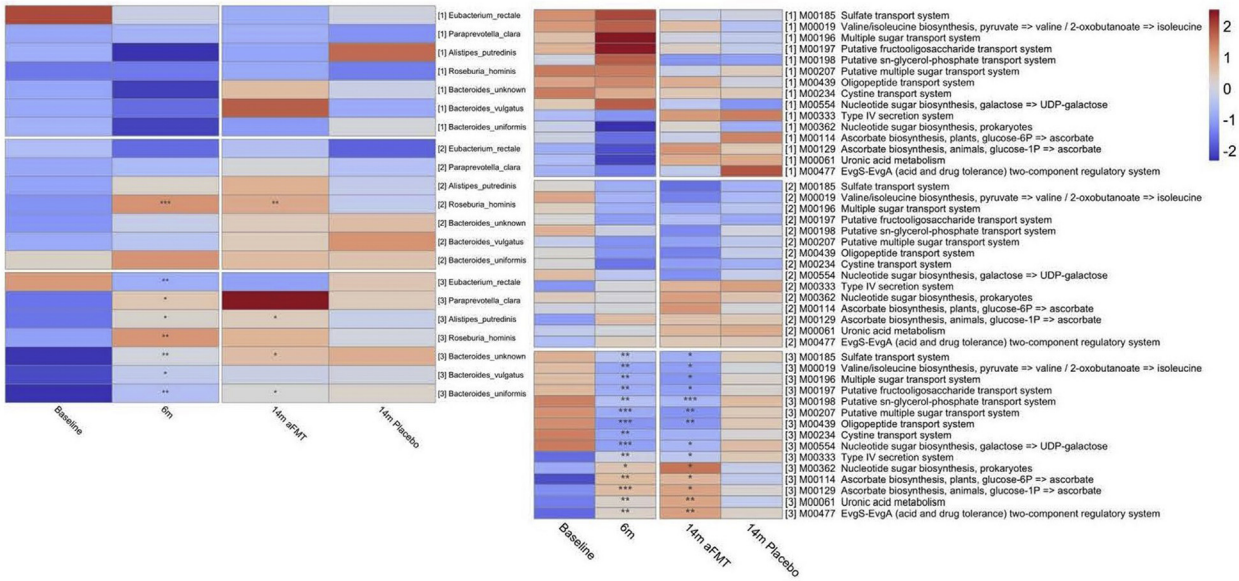
Supplementary Figure 3. 0 to 6-month KEGG modules changes across lifestyle intervention arms. KEGG modules that were significantly changed by weight loss, across lifestyle intervention groups. Values represents log2 fold-change from baseline, by module. No significant changes were observed among the Healthy dietary guidance group.



Supplementary Figure 4. Shotgun metagenomics processing validation against MetaPhlan2. (*A*) Correlation between pairwise samples dissimilarity, processed by BURST vs MetaPhlan2, for all possible pairs of sequenced samples. (*B*) Comparison between the number of observed aFMT-associated taxa, generated by MetaPhlan2, to the number expected by a permuted null model by an iterative randomization (n = 1000) of sample labeling across group, treatment and time. The *gray dot* denotes the observed number of taxa in each group, while the violins enclose 95% of the permuted results. *P* value was calculated as observed/expected.



Supplementary Figure 5. Microbiome taxonomic and functional changes, centered-log-transformed data. Shotgun metagenomics assessed bacteria and metabolic pathways (KEGG modules) after centered log transformation that were significantly changed by weight loss and maintained by the aFMT treatment, across lifestyle intervention groups.



Supplementary Figure 6. Microbiome taxonomic and functional changes, excluding subjects with prescribed metformin. Shotgun metagenomics assessed bacteria and metabolic pathways (KEGG modules), excluding subject with prescribed metformin, that were significantly changed by weight loss and maintained by the aFMT treatment, across lifestyle intervention groups.

Supplementary Table 1. Outline of Dietary and Physical Activity Interventions

Variable	Healthy dietary guidance	Mediterranean diet	Green-Mediterranean diet
Physical activity	18 mo free gym membership 18 mo PA education sessions 45–60 min of aerobic training + resistance training, 3–4 times/wk	18 mo free gym membership 18 mo PA education sessions 45–60 min of aerobic training + resistance training, 3–4 times/wk	18 mo free gym membership 18 mo PA education sessions 45–60 min of aerobic training + resistance training, 3–4 times/wk
Lifestyle group sessions	18 mo group sessions in the workplace, weekly for the first month, and monthly thereafter	18 mo group sessions in the workplace, weekly for the first month, and monthly thereafter	18 mo group sessions in the workplace, weekly for the first month, and monthly thereafter
General dietary guidance	Limit dietary cholesterol, *trans* fat, saturated fat, sugars, and salt and increase intake of vegetables	Limit dietary cholesterol, *trans* fat, saturated fat, sugars, and salt and increase intake of vegetables	Limit dietary cholesterol, *trans* fat, saturated fat, sugars, and salt and increase intake of vegetables
Energy, kcal/d	Guidelines for a healthy Mediterranean diet with no specific recipes or calorie restriction	1500–1800 kcal/d for men, 1200–1400 kcal/d for women	1500–1800 kcal/d for men, 1200–1400 kcal/d for women
Total fat, % of daily consumption	Guidelines for a healthy Mediterranean diet with no specific recipes or calorie restriction	Approximately 40% mainly PUFA and MUFA	Approximately 40% mainly PUFA and MUFA
Carbohydrates, g/d	Guidelines for a healthy Mediterranean diet with no specific recipes or calorie restriction	<40 g/d in the first 2 mo with increased gradual intake up to 80 g/d	<40 g/d in the first 2 mo with increased gradual intake up to 80 g/d
Specific recommendations	Guidelines for a healthy Mediterranean diet with no specific recipes or calorie restriction	Avoid red and processed meats. Reduced poultry intake	Avoid red and processed meats. Reduced poultry intake
Polyphenols, mg/d	Guidelines for a healthy Mediterranean diet with no specific recipes or calorie restriction	+440 mg/d (source: provided walnuts [28 g/d])	+1240 mg/d (source: provided walnuts [28 g/d]), green tea [4 cups/d], *Wolffia globosa* duckweed [Mankai] shake [100 g frozen cubes])

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Supplementary Table 2. Chronic Pharmacotherapy of the Participants at Baseline

	All subjects			Healthy dietary guidance group			Mediterranean diet			Green-Mediterranean diet		
Treatment	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value
Subjects, n	44	46		8	8		17	18		19	20	
Oral antihyperglycemic, n (%)	3 (6.8)	3 (6.5)	1	1 (12.5)	0 (0.0)	1	0 (0.0)	1 (5.6)	1	2 (10.5)	2 (10.0)	1
Exogenous insulin, n (%)	1 (2.3)	1 (2.2)	1	0 (0)	0 (0)	1	0 (0)	0 (0)	1	1 (5.3)	1 (5.0)	1
Antihypertensive, n (%)	10 (22.7)	9 (19.6)	.91	3 (37.5)	0 (0.0)	.2	2 (11.8)	4 (22.2)	.71	5 (26.3)	5 (25.0)	1
Cholesterol-lowering, n (%)	6 (13.6)	8 (17.4)	.84	2 (25.0)	1 (12.5)	1	1 (5.9)	1 (5.6)	1	3 (15.8)	6 (30.0)	.5
Antiplatelet, n (%)	6 (13.6)	2 (4.3)	.24	2 (25.0)	0 (0.0)	.45	1 (5.9)	0 (0.0)	.98	3 (15.8)	2 (10.0)	.95

Supplementary Table 3. Characteristics of the Study Population at 14 Months

	All subjects			Healthy dietary guidance group			Mediterranean diet			Green-Mediterranean diet		
Variable	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value
Subjects, n	44	46		8	8		17	18		19	20	
Body mass index, kg/m^2, mean (SD)	28.92 (3.12)	29.53 (4.21)	.82	29.11 (3.51)	28.31 (1.72)	.73	29.23 (3.69)	29.75 (4.96)	.77	28.55 (2.46)	29.79 (4.16)	.42
Waist circumference, *cm*, mean (SD)	101.45 (7.85)	102.25 (10.84)	.94	100.00 (9.62)	97.00 (4.62)	.86	102.47 (8.66)	103.94 (11.80)	.84	101.16 (6.52)	102.58 (11.33)	.75
Weight, *kg*, mean (SD)	87.69 (12.58)	86.92 (14.25)	.80	86.15 (14.90)	79.27 (8.57)	.25	87.99 (13.48)	89.63 (15.78)	.70	88.07 (11.35)	87.18 (13.98)	.91
Fasting plasma glucose, *mg/dL*, mean (SD)	103.57 (14.69)	99.78 (9.18)	.29	104.18 (17.13)	98.48 (6.74)	.73	99.28 (7.41)	98.89 (10.65)	.27	107.17 (18.05)	101.17 (8.80)	.52
Fasting plasma insulin, $\mu U/mL$, mean (SD)	10.16 (4.61)	11.54 (7.05)	.56	10.95 (4.40)	11.34 (7.38)	.83	10.06 (3.84)	10.89 (5.91)	.97	9.92 (5.46)	12.21 (8.08)	.42
HOMA-IR, mean (SD)	2.55 (1.29)	2.88 (1.86)	.69	2.53 (1.06)	2.83 (1.98)	.73	2.46 (0.93)	2.67 (1.55)	1	2.65 (1.65)	3.11 (2.14)	.63

NOTE. No significant differences were observed between placebo or aFMT group in the measured baseline characteristics, overall and across lifestyle interventions. HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

Supplementary Table 4. Adherence to Diet at Time 6 Months; Adherence to Physical Activity at Baseline and 6 Months

Adherence to dietary intervention at 6 mo	Healthy dietary guidance	Mediterranean diet	Green-Mediterranean diet	*P* value
% Carbohydrates intake	45.99 (8.13)	32.53 (6.88)	37.19 (8.53)	<.001
% Protein intake	18.97 (3.58)	27.02 (4.23)	23.21 (4.65)	<.001
% Fat intake	36.41 (5.13)	41.15 (6.27)	40.76 (7.25)	.1
Adherence to physical activity intervention				
Baseline METs/wk	38.97 (48.68)	42.08 (18.62)	35.08 (22.98)	.723
6-mo METs/wk	56.72 (47.17)	72.67 (41.39)	51.36 (33.36)	.212

NOTE. Values are presented as mean (SD). Proportional macronutrient intake (6-mo time point) and METs (baseline and 6-mo time point) across lifestyle intervention group. METs unit are defined as the ratio of work metabolic rate to the standard resting metabolic rate.

Supplementary Table 5. Adherence to Diet and Physical Activity Across Lifestyle Intervention Arms and Treatment Groups at the End of the Trial

	Healthy dietary guidance			Mediterranean diet			Green-Mediterranean diet		
Variable	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value	aFMT	Placebo	*P* value
% Carbohydrates intake	43.90 (6.01)	44.62 (2.05)	.855	38.01 (6.34)	36.94 (8.47)	.727	41.85 (6.55)	39.13 (8.26)	.447
% Protein intake	19.99 (1.76)	20.59 (1.14)	.584	23.91 (3.14)	22.98 (4.79)	.6	20.86 (2.23)	20.82 (3.45)	.861
% Fat intake	37.22 (5.09)	35.44 (2.17)	.715	38.98 (4.59)	41.63 (5.46)	.116	38.74 (5.58)	40.48 (5.67)	.482
METs/wk	67.58 (49.77)	55.91 (54.07)	.624	46.27 (26.98)	48.69 (34.80)	.93	36.14 (31.75)	33.86 (27.19)	.804

NOTE. Values are presented as mean (SD). Proportional macronutrient intake and METs across lifestyle intervention group and aFMT treatment at the end of the trial. METs unit are defined as the ratio of work metabolic rate to the standard resting metabolic rate.