Original research

Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial

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ABSTRACT

Objective The impact of faecal microbiota transplantation (FMT) on microbiota engraftment in patients with metabolic syndrome is uncertain. We aimed to study whether combining FMT with lifestyle modification could enhance the engraftment of favourable microbiota in obese patients with type 2 diabetes mellitus (T2DM).

Design In this double-blind, randomised, placebo-controlled trial, 61 obese subjects with T2DM were randomly assigned to three parallel groups: FMT plus lifestyle intervention (LSI), FMT alone, or sham transplantation plus LSI every 4 weeks for up to week 12. FMT solution was prepared from six healthy lean donors. Faecal metagenomic sequencing was performed at baseline, weeks 4, 16 and 24. The primary outcome was the proportion of subjects acquiring ≥20% of microbiota from lean donors at week 24.

Results Proportions of subjects acquiring ≥20% of lean-associated microbiota at week 24 were 100%, 88.2% and 22% in the FMT plus LSI, FMT alone, and sham plus LSI groups, respectively ($p<0.0001$). Repeated FMTs significantly increased the engraftment of lean-associated microbiota ($p<0.05$). FMT with or without LSI increased butyrate-producing bacteria. Combining LSI and FMT led to increase in *Bifidobacterium* and *Lactobacillus* compared with FMT alone ($p<0.05$). FMT plus LSI group had reduced total and low-density lipoprotein cholesterol and liver stiffness at week 24 compared with baseline ($p<0.05$).

Conclusion Repeated FMTs enhance the level and duration of microbiota engraftment in obese patients with T2DM. Combining lifestyle intervention with FMT led to more favourable changes in recipients' microbiota and improvement in lipid profile and liver stiffness.

Trial registration number NCT03127696.

Significance of the study

What is already known on this subject?

- A single faecal microbiota transplantation (FMT) alone led to modest engraftment of donors' microbiota in obese subjects with metabolic syndrome.
- Lifestyle affects the microbiota composition in both animal and humans.
- Dietary manipulation may improve body weight and glycaemic control through alternation of gut microbiota.

What are the new findings?

- FMT repeated at scheduled intervals led to increased and sustained engraftment of microbiota irrespective of lifestyle modification.
- Combining lifestyle intervention with FMT led to favourable changes in recipients' microbiota and improvement in lipid profile compared with either intervention alone.

How might it impact on clinical practice in the foreseeable future?

- Repeated FMTs are safe and lead to increased and sustained engraftment of donors' microbiota in patients with obesity and diabetes. Adding lifestyle intervention to FMT further improves recipients' microbiota profile and reduces low-density lipoprotein cholesterol. Refining donor selection criteria and metagenomic profile of lean microbiota render FMT as a potential therapy for metabolic syndrome in the future.

INTRODUCTION

There is a worldwide epidemic of obesity and type 2 diabetes mellitus (T2DM). Conventional non-pharmacological interventions based on diet and exercise showed limited success in achieving sustained weight loss.[1] Growing evidence supports the role of enteric microbiota in the pathogenesis of obesity-related insulin resistance and T2DM.

Studies have reported that obese individuals had abnormal gut microbiota[2,3] and humanised mouse models suggest that the gut microbiota may be a causative factor in obesity.[4] Patients with T2DM are also characterised by dysbiosis with a decrease in abundance of butyrate-producing bacteria and enrichment of microbial functions conferring sulphate reduction and oxidative stress resistance.[5] The obese microbiome appeared to be more efficient in harvesting energy from diet.[6]

Faecal microbiota transplantation (FMT) offers hope to restore the gut microbial ecology. Early pilot data suggested that FMT improves insulin sensitivity and increases gut microbial diversity accompanied by a distinct increase in butyrate-producing bacteria in patients with metabolic syndrome.[7,8] Two small-scale randomised trials showed that administration of oral microbiota capsules to obese subjects without T2DM resulted in short-term engraftment of donors' microbiota.[9,10] A recent randomised trial in type 1 diabetes mellitus showed that FMT could halt the decline in endogenous insulin production and certain gut microbiota were linked to preserved beta cell function in humans.[11] However, two important questions remain unclear. First, it is uncertain whether FMT can lead to sustainable microbiota engraftment. The latter is a prerequisite for FMT to be considered as a potential option for the treatment of obesity and metabolic syndrome. Second, it is also unclear whether dietary and lifestyle modification will confer additional benefits to FMT such as enhancing microbiota engraftment, correcting biochemical parameters and improving clinical outcomes. Interestingly, cohousing mice harbouring obese microbiota with mice harbouring lean microbiota prevented weight gain in obese microbiota containing mice, suggesting that diet and lifestyle may influence gut microbiota.[12] In addition, autologous FMT, collected during diet-induced weight loss phase and administrated in the weight regain phase, has been shown to sustain weight loss and glycaemic control.[13] In this randomised controlled trial, we investigated the long-term effects of repeated FMTs with or without lifestyle modification on the engraftment of lean donors' microbiota in obese subjects with T2DM. We hypothesise that combining lifestyle intervention with repeated FMTs would improve microbiota engraftment in obese recipients with T2DM.

MATERIALS AND METHODS

Study design and participants

This was a randomised, double-blind, placebo-controlled trial in obese Chinese subjects with T2DM. Obesity for Asian populations is defined as a BMI of 25 kg/m^2 or higher.[14] Obese subjects aged 18–70 years with a body mass index of $\geq 28\,kg/m^2$ and $<45\,kg/m^2$ and a diagnosis of T2DM for at least 3 months were recruited from a tertiary referral centre in Hong Kong. Patients with the use of weight loss medication in the preceding 1 year, immunodeficiency syndrome, contraindications to oesophago-gastro-duodenoscopy (OGD), history of food allergies, severe organ failure including decompensated cirrhosis, IBD, kidney failure, epilepsy, known malignancy in recent 2 years and active sepsis were excluded. Subjects taking antibiotics or probiotics within 12 weeks of screening, or those on sodium-glucose co-transporter-2 inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists or proton-pump inhibitors at randomisation were also excluded. Antibiotics, probiotics or prebiotics were prohibited during study period (online supplemental appendix). During the study, patients remained on the same dose of oral hypoglycemic drugs and lipid-lowering drugs. All patients gave written informed consent.

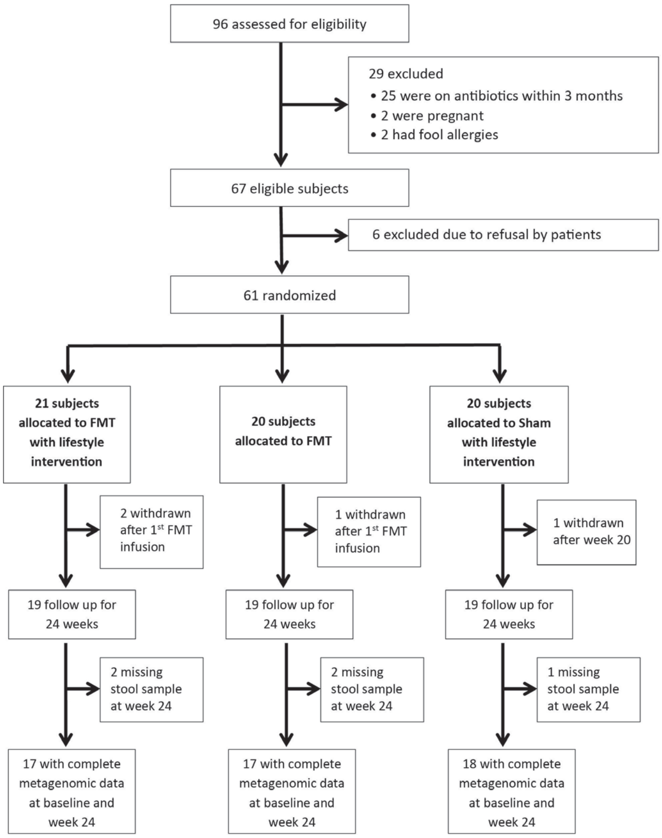

Figure 1 Consort diagram. FMT, faecal microbiota transplantation.

Randomisation and masking

Patients were randomly assigned (1:1:1) to three parallel groups: FMT with lifestyle intervention (LSI), FMT alone, or sham transplantation with LSI every 4 weeks for up to week 12. Randomisation code was computer generated and performed in blocks of 12. Subjects were randomised in sequence according to the randomisation code and arranged to have infusion according to a randomisation table (figure 1). Only physicians and staff who were blinded to the study assignment were involved in follow-up visits.

Procedures

We screened potential donors with a body mass index (BMI) $<23\,kg/m^2$ according to a set of stringent criteria (online supplemental appendix). Stool samples of eligible donors were collected within 4 hours of bowel movements, which were inspected visually for suitability (formed stool, no blood or mucus). Donor stool was homogenised with isotonic saline and glycerol, filtered and then stored at −80°C. FMT solutions were prepared from pooling of donors' stool. Sham transplantation was done by saline infusion. All FMT and sham solutions were wrapped in opaque foil for blinding. We infused 100–200 mL of FMT solution with 50 g stool or sham solution over 2–3 min into the distal duodenum via OGD under conscious sedation.

Follow-up assessment

Patients were followed up every 4 weeks until week 24. Subjects in FMT plus LSI arm or sham transplantation plus LSI arm were seen by a dietitian at weeks 0, 1, 2, 4, 6, 8 and 12. The dietitian provided comprehensive assessment and individualised advice on diet, lifestyle and behaviour to facilitate weight loss. All subjects recorded daily physical activity and completed 3-day dietary records at weeks 0, 4, 8, 12 and 24. Details of lifestyle intervention are shown in online supplemental appendix.

Faecal microbiota analysis using metagenomic sequencing

We collected blood samples at baseline and week 24, and stool samples at baseline, weeks 4, 16 and 24. Faecal DNA for bacterial metagenomics sequencing was isolated using Maxwell RSC PureFood GMO and Authentication Kit according to the manufacturer's instructions. DNA libraries were constructed through the processes of end repairing, purification and PCR amplification, and sequenced by Illumina Novaseq 6000 with paired-end 150 bp sequencing strategy by Novogene, Beijing, China, generating 93.5±15.2 million (mean±SD) raw reads per sample.

Metagenomics reads were quality-filtered and trimmed using Trimmomatic[15] (V.0.38) and decontaminated against human genome (Reference: hg38) by Kneaddata (V.0.7.2, https://bitbucket.org/biobakery/kneaddata/wiki/Home). For bacteriome, MetaPhlAn2[16] (V.2.6.0) was used to generate a species-level metagenomic analysis. Functional analysis was performed by HUMANN2[17] (V.0.11.1). GNU parallel (Tange O. 2018) was used for parallel analysis jobs to accelerate data processing. The resulting abundance tables were processed in R V.3.6.0 and tidyverse[18] (V.1.2.1), ggpubr[19] (V.0.2) R packages. The abundance table was normalised by centred log-ratio transformation and phyloseq[20] (V.1.24.2) R package before calculating principal coordinates analysis (PCoA) using Bray-Curtis distance. Growth rate index (GRiD) was used to infer bacterial replication rate as previously described.[21] Faecal metabolomic sequencing were analysed using ultra performance liquid chromatography (ExionLC AD) and tandem mass spectrometry (QTRAP) as previously described.[22]

Outcomes

The primary outcome was the proportion of subjects acquiring least 20% of microbiota from lean donors after FMT at week 24. A previous study observed that FMT led to engraftment of an average of 10% lean-associated microbiota in subjects with metabolic syndrome after one single dose of FMT.[23] We considered that 10% engraftment was modest and that FMT is potentially useful for the treatment of obesity if we could further enhance the efficacy of microbial engraftment. We therefore arbitrarily chose 20% as the cut-off because we considered repeated FMTs would be superior to a single FMT if the former approach could achieve at least twice the level of microbiota engraftment compared with a single FMT.[23] Species were considered present if the relative abundance was greater than or equal to 0.01%. We classified microbiota species identified in the recipients into four types, namely, (1) 'lean-associated' if it was absent in the recipient baseline sample but present in both the corresponding donor and post-FMT sample; (2) 'recipient-exclusive' if it was present in both recipient baseline and post-FMT samples but absent in the corresponding donor; (3) 'co-existed' if it was present in recipient baseline sample, post-FMT sample and the corresponding donor; and (4) 'unknown' if it was absent in both recipient baseline sample and corresponding donor sample but was present in post-FMT sample of the recipient.[23] Secondary outcomes were safety, proportion of subjects achieving at least 10% weight reduction at week 24 and change in metabolic parameters including total cholesterol, low-density lipoprotein (LDL) cholesterol, fasting glucose, fasting insulin, haemoglobin A1c, liver stiffness and liver controlled attenuation parameter (CAP) measured on transient elastography at week 24. Safety was assessed based on adverse events.

Statistical analysis

Our sample size estimation was based on the assumption that FMT for obesity would achieve at least 20% lean-associated microbiota in 80% of recipients whereas only 20% of patients using lifestyle intervention would achieve this target. Assuming that 30% of patients would be lost to follow-up, a total of 60 evaluable cases would be required to achieve a power of 90% at 5% level of significance (Fisher's exact test). The intention-to-treat analysis included all patients who received at least one study dose. Continuous variables were expressed in mean±SD or median (25th to 75th percentile, P25–P75) as appropriate, whereas categorical variables were presented as number (percentage). Fisher's exact test was performed for comparisons between proportions. Repeated measures ANOVA (with logarithmic transformation for skewed variables) was applied for more than two-occasion and between-group comparisons. If the time-by-group interactions of the repeated measures ANOVA were significant, individual Kruskal-Wallis test was used to further investigate significant differences between treatment arms at the same time. This was followed by Mann-Whitney U test with Bonferroni correction for multiple comparisons. Wilcoxon signed-rank test was used to compare data between different time points within the same treatment. P values were adjusted for multiple analyses. Paired t-test or Wilcoxon signed-rank test was used to compare data between the baseline and week 24 within the same treatment where appropriate. Linear discriminant analysis effect size[24] (LEfSe) was used to determine the taxa between two groups. Differences between pre-FMT and post-FMT samples were assessed by Euclidean distance after applying a logarithmic transformation to relative abundances using the function $\log_{10}(x+x_0)$, where x was the original relative abundance and $x_0=1e^{-4}$. All statistical tests were two-sided. Statistical significance was taken as p value <0.05.

RESULTS

From 26 April 2017 to 29 November 2018, 90 obese subjects with T2DM were screened for eligibility. Among them, 61 subjects were randomly allocated to either FMT plus LSI (n=21), FMT alone (n=20), or sham transplantation plus LSI (n=20). Fifty-seven subjects received at least one dose of treatment and completed the final follow-up at 24 weeks. Fifty-three of 57 subjects had complete metagenomic data at baseline and 24 weeks (figure 1 and online supplemental figure 1). Demographic characteristics of the three study groups were comparable (table 1). Majority of the participants were male (71%), with median BMI of 30.2, 32.8 and 33.4 kg/m^2, in the FMT plus LSI, FMT alone, or sham plus LSI groups, respectively. The median HbA1c was 7.1%, 7.4% and 7.7% in the FMT plus LSI, FMT alone, or sham plus LSI groups, respectively. Six healthy lean donors (67% male, median BMI: 20.1 kg/m^2 (P25–P75: 19.3–21.6 kg/m^2)) provided stool for the FMT solution. Twelve (63.2%) and ten (53.2%) subjects in FMT plus LSI and sham plus LSI groups, respectively, achieved <50% of the dietary and lifestyle goals. There was no significant difference in total calorie intake at baseline, week 12 and week 24 in all three groups. Baseline characteristics of the FMT donors are shown in online supplemental table 1).

Repeated FMT at scheduled intervals led to sustained engraftment of microbiota irrespective of lifestyle modification

The difference between lean donors and recipient baseline was dominated by enriched *Faecalibacterium* and *Roseburia*, both

Table 1 Subject baseline characteristics by treatment groups

Total (n=61)	FMT with lifestyle intervention (n=21)	FMT (n=20)	Sham with lifestyle intervention (n=20)
Male, n (%)	15 (71.4)	14 (70.0)	14 (70.0)
Age, years (P25–P75)	56 (45.5–64.5)	55.5 (44.3–61.8)	55.0 (47.5–62.5)
Smoker/ex-smoker, n (%)	8 (38.1)	6 (30.0)	4 (20.0)
Alcoholic drinker/ex-drinker, n (%)	5 (23.8)	1 (5.0)	1 (5.0)
T2DM diagnosis, years (P25–P75)	8.7 (4.2–10.9)	7.2 (2.9–12.2)	4.8 (1.5–12.4)
Body weight, kg (P25–P75)	95 (74–106)	90.7 (76.5–99.1)	93.2 (82.7–97.9)
BMI, kg/m^2 (P25–P75)	30.2 (28.8–34.6)	32.8 (29.8–36.5)	33.4 (31.9–36.2)
Systolic blood pressure, mm Hg (P25–P75)	136 (126–143)	138 (118–146)	134 (124–146)
Diastolic blood pressure, mm Hg (P25–P75)	79 (70–87)	76 (71–84)	77 (72–84)
Waist:hip ratio (P25–P75)	0.95 (0.92–1.03)	0.996 (0.98–1.03)	0.97 (0.95–1.06)
Fasting glucose, mmol/L (P25–P75)	6.6 (5.6–7.4)	6.2 (5.2–7.4)	6.2 (5.3–6.7)
Fasting insulin, mIU/L (P25–P75)	20 (13–34)	17 (13–29)	19 (15–31)
HbA1c, % (P25–P75)	7.1 (6.7–8.6)	7.4 (6.2–8.5)	7.7 (6.5–8.7)
HOMA-IR (P25–P75)	0.9 (0.8–1.0)	0.9 (0.8–1.0)	0.85 (0.7–0.9)
Cholesterol			
Total, mmol/L (P25–P75)	4.2 (3.7–5.0)	4.5 (3.6–4.9)	4.0 (3.5–4.4)
LDL, mmol/L (P25–P75)	2.3 (1.5–2.9)	2.2 (1.7–2.5)	1.7 (1.4–2.3)
HDL, mmol/L (P25–P75)	1.4 (1.2–1.5)	1.2 (1.1–1.5)	1.3 (1.0–1.5)
Triglycerides, mmol/L (P25–P75)	1.6 (1.2–1.9)	1.8 (1.1–2.6)	1.5 (1.2–2.7)
ALT, IU/L (P25–P75)	26 (20–40)	36 (21–49)	32 (21–47)
Liver stiffness, kPa (P25–P75)	5.3 (4.7–7.8)	7.3 (5.8–8.2)	7.7 (5.2–10.7)
Liver CAP, dB/m (P25–P75)	341 (297–360)	342 (278–374)	334 (304–379)

Data expressed as number of subjects (%) or median (P25–P75).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus.

belonging to fibre-degrading and butyrate-producing genera (online supplemental table 2). Donor-derived bacterial species were detected in post-FMT samples from all recipients. For the primary outcome, among 53 subjects who had completed 24 weeks of follow-up and complete stool metagenomic sequencing data, 100% in the FMT plus LSI group, 88.2% in the FMT alone group and 22.2% in the sham plus LSI arm achieved ≥20% lean-associated microbiota at week 24 ($p<0.001$, Fisher's exact test, figure 2A). The percentage of lean-associated microbiota in both FMT alone and FMT plus LSI groups were significantly higher than sham plus LSI group after intervention ($p<0.0001$, repeated measures ANOVA, figure 2B). No significant difference was observed between FMT alone and FMT plus LSI groups. At week 24, the percentage of lean-associated microbiota in FMT plus LSI group, FMT alone group and sham plus LSI group were 29.7% (26.4%–35.4%), 29.0% (26.0%–30.8%) and 13.6% (9.2%–18.6%) (median, P25–P75), respectively. Subgroup analysis between subjects with different compliance to lifestyle modification showed no significant difference in the percentage of microbiota acquired from lean donors (online supplemental figure 2).

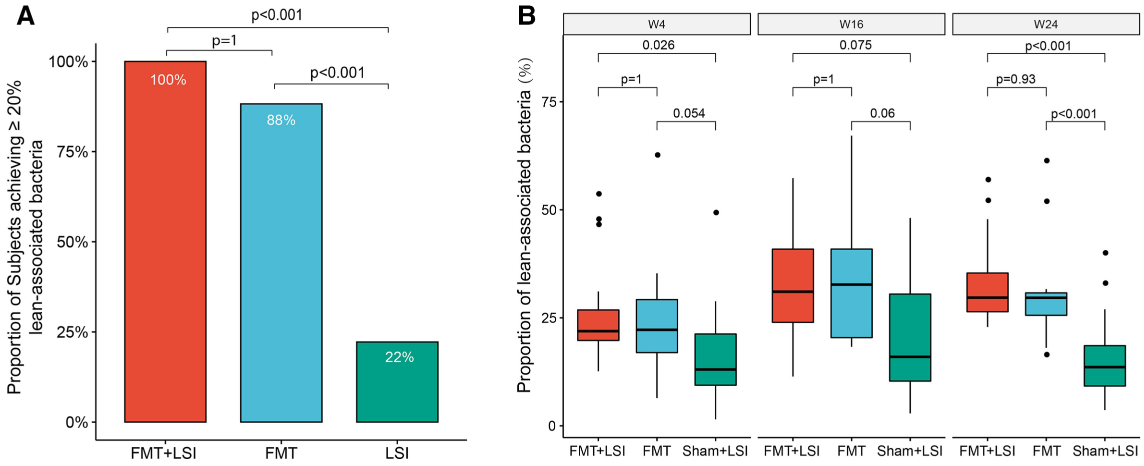

Figure 2 Faecal microbiota analysis showing FMT led to durable engraftment of lean-associated microbiota in obese subjects with type 2 diabetes. (A) Proportion of subjects with ≥20% lean-associated microbiota at W24. (B) Boxplot depicting of the percentage of lean-associated microbiota at W4, W16 and W24. Significance between treatment arms were calculated by Mann-Whitney U test with Bonferroni correction. FMT, faecal microbiota transplantation; LSI, lifestyle intervention.

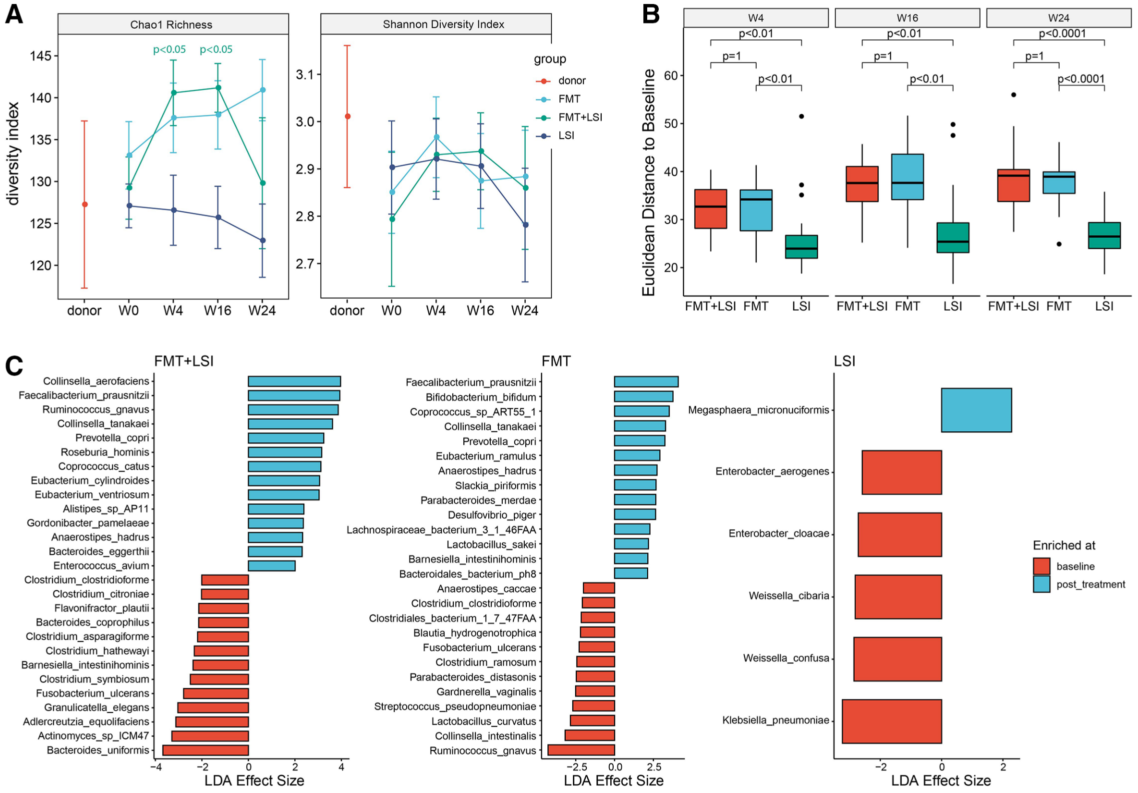

Figure 3 Faecal microbiota analysis showing FMT conferred more favourable changes than sham with lifestyle Intervention. (A) Changes in microbiota richness and diversity represented by Chao1 richness and Shannon diversity index after intervention in three treatment arms. Significance within treatment arms were calculated by Wilcoxon signed-rank test. (B) Changes of Euclidean distance between baseline and post-treatment microbiota composition at W4, W16 and W24. Significance between treatment arms were calculated by Mann-Whitney U test with Bonferroni correction. (C) LDA effect size of bacterial species that significantly altered after intervention in each treatment arm by LEfSe (LDA>2, adjusted p<0.05). FMT, faecal microbiota transplantation; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size.

The percentage of lean-associated microbiota in both the FMT alone and FMT plus LSI groups were significantly higher at weeks 16 and 24 compared with week 4 ($p<0.01$ and $p<0.05$, respectively, Wilcoxon signed-rank test), suggesting multiple times of FMT significantly increased the percentage of lean-associated microbiota in FMT alone and FMT plus LSI groups.

FMT with lifestyle intervention led to significant increase of bacterial richness

While there was no significant change in bacterial diversity between treatment groups, bacterial richness significantly increased in FMT plus LSI arm at week 4 and week 16 after intervention ($p<0.01$ and $p<0.05$, Wilcoxon signed-rank test, figure 3A). No significant alteration in bacterial richness was seen in the sham plus LSI group. Bacterial richness in the FMT groups were significantly higher than sham plus LSI group after treatment ($p<0.0001$, repeated measures ANOVA).

FMT led to increase of butyrate-producing bacteria and enrichment in related pathways

The Euclidean distance between baseline and post-treatment samples reflects the changes in overall microbiota composition before and after intervention. The Euclidean distance between baseline and post-treatment samples in FMT alone and FMT plus LSI groups were significantly higher than that of sham plus LSI group ($p<0.001$, repeated measures ANOVA, figure 3B), suggesting that FMT and FMT plus LSI induced more changes in overall microbiota composition than sham plus LSI. Concurrently, the relative abundance of 27, 26 and 6 bacterial species were significantly altered in FMT plus LSI group, FMT alone and sham plus LSI alone group post-intervention, respectively (LDA >2, adjusted $p<0.05$). Subjects in both FMT groups had a significant increase in *Prevotella copri* and several butyrate-producing bacteria, including *Faecalibacterium prausnitzii, Collinsella tanakaei, Anaerostipes hadrus*, several *Eubacterium* spp. and *Coprococcus* spp., and reduction of *Clostridium clostridioforme* and *Fusobacterium ulcerans*, which were not seen in the sham plus LSI group (figure 3C). The species with highest engraftment rate were *Collinsella tanakaei* and *Roseburia hominis* (online supplemental file). Following treatments, the butyrate-producing bacteriome profile in FMT groups resembled that of the donors while sham plus LSI group showed no obvious shift in bacteria (online supplemental figure 3). After intervention, subjects in FMT plus LSI group were enriched with *Bifidobacterium* spp. and *Lactobacillus* spp. compared with FMT alone group (LDA >2, adjusted $p<0.05$, online supplemental figure 4). We then inferred bacterial replication rate (GRiD) from metagenomic sequencing. The most altered species such as *F. prausnitzii*, *A. hadrus* and *R. hominis* were viable (GRiD>1) in at least one of the donors (online supplemental figure 5A). The GRiD values of *F. prausnitzii* and *R. hominis* were significantly increased in FMT recipients at week 24 compared with baseline ($p<0.05$, Wilcoxon signed-rank test, online supplemental figure 5B). The relative abundance of several microbiota functional pathways was increased after FMT, compared with their respective baseline samples. Faecal microbiota in the

FMT alone and FMT plus LSI groups showed decreased level of amino acid degradation, secondary metabolite degradation, and increased level of secondary metabolite biosynthesis, non-carbon nutrient degradation, L-ornithine biosynthesis, carbohydrate degradation, vitamin biosynthesis, nucleotide degradation and amino acid biosynthesis, compared with their baseline faecal samples (LDA >2, adjusted $p<0.05$). Comparing with baseline, the abundance of butyric acids in both FMT alone and FMT plus lifestyle arms increased while the abundance of acetate and propionate decreased, but the difference did not reach statistical significance (online supplemental figure 6). In the sham plus LSI group, no significant changes in microbiota functional pathways were observed.

FMT with lifestyle intervention led to reduced LDL and liver stiffness

For the secondary outcomes, two of 41 subjects (4.9%) in the FMT groups and none of 20 (0%) in the sham plus LSI group achieved ≥10% weight loss at week 24. Among the three treatment groups, subjects in the FMT plus LSI group had a significant decrease in total cholesterol ($p=0.046$) and low-density lipoprotein (LDL) cholesterol level ($p=0.011$) at week 24 compared with FMT alone (total cholesterol: $p=0.837$; LDL: $p=0.955$) and sham plus LSI groups (total cholesterol: $p=0.333$; LDL: $p=0.275$). The effect of this reduction in LDL cholesterol level was sustainable at week 24 (online supplemental figure 7). Subjects who had FMT and LSI also had a reduction in their total and LDL cholesterol at week 24 compared with baseline ($p=0.05$ and $p=0.04$, respectively). There was a significant decrease in liver CAP score in FMT+LSI group at week 24 compared with baseline ($p=0.013$), but not in the other two groups (online supplemental table 3). Subgroup analysis showed that subjects who achieved ≥50% of the dietary and lifestyle goals had significantly more weight loss and improved liver CAP score at week 24 compared with subjects who achieved <50% of the dietary and lifestyle goals in FMT+LSI arm ($p<0.05$, Wilcoxon rank sum test, online supplemental figure 8). No significant difference was observed in the proportion of subjects on lipid-lowering agents in all treatment groups at baseline and at week 24. There was also no significant difference in fasting glucose level, fasting insulin level and HbA1c in each group at week 24 compared with baseline except in the group of sham with LSI, and there was a significant increase in fasting glucose level at week 24 compared with baseline.

Adverse events following FMT in obese patients with T2DM

The most commonly reported adverse events (AEs) were GI upset, which was comparable between the three arms (33.3%, 40%, 35%) (online supplemental table 4). None of the subjects with AEs were hospitalised and all recovered spontaneously. Five subjects in the FMT groups (two in FMT plus LSI and three in FMT alone groups) experienced serious AEs (one known history of supraventricular tachycardia undergoing scheduled electrophysiological study with radiofrequency ablation at week 8, one incidental finding of paroxysmal atrial flutter at week 11, one atypical chest pain with negative cardiac workup at week 11, one with known ischaemic heart disease developed acute coronary syndrome at week 20, one with known psoriasis and diabetes mellitus developed pelvic inflammatory disease at week 23). One subject in the sham plus LSI group developed left carpal tunnel syndrome requiring operation at week 24. None of the AEs were considered to be related to intervention. There was no mortality.

DISCUSSION

This study aimed to explore whether combining FMT with lifestyle modification could enhance the engraftment of lean donors' microbiota in obese patients with T2DM. Enhancing microbiota engraftment is a prerequisite for FMT to be a potentially useful treatment for metabolic syndrome. Our study has, for the first time, demonstrated that FMT repeated at scheduled intervals led to increased and sustainable engraftment of microbiota from lean donors in obese recipients with T2DM that persisted for at least 6 months. We found that although combining lifestyle modification with FMT did not further increase the engraftment of lean-associated microbiota, this combined strategy led to a significant increase in bacterial richness and enrichment of probiotic species, and a favourable reduction in cholesterol level and liver fibroscan scores.

Comparing with previous studies,[9 10 23] our FMT protocol led to more substantial engraftment of lean donors' microbiota both in terms of proportion and number of engrafted taxa. Two factors account for our favourable results. First, we used repeated FMTs at regular intervals for up to 3 months instead of a single FMT. At week 24, recipients harboured about 30% of lean-associated microbiota whereas a previous study using a single FMT showed only 10% of engraftment.[23] Second, we used multi-donor FMT to minimise the potential risk of a single ineffective donor and to enhance the microbial diversity transferred to recipients.[25] A previous study reported highly variable level of microbiota engraftment with a single donor.[9] Third, we chose duodenal delivery because the small intestine microbiota has been shown to regulate host digestive and absorption of dietary lipids.[26] Duodenal delivery has an additional advantage of not requiring bowel preparation.

Our study has provided a new perspective on the role of lifestyle intervention in obese patients with T2DM undergoing FMT. Lifestyle intervention did not enhance microbiota engraftment after FMT. Nevertheless, FMT plus LSI led to more favourable changes in microbiota than FMT alone, as evidenced by increased bacterial richness and enriched probiotic *Bifidobacterium* spp. and *Lactobacillus* spp. Notably, only FMT plus LSI group had a significant reduction in total and LDL cholesterol whereas LSI alone had no such effect, suggesting that FMT enhances lipid metabolism in the presence of lifestyle intervention. Microbiota has been shown to regulate host dietary lipid digestion and absorption in animal experiments.[26]

Consistent with previous studies, we found that FMT increases multiple butyrate-producing bacteria and enriches pathways of carbohydrate degradation.[9 10] Butyrate could also act directly as a substrate for adipogenesis in adipose tissues, thereby reducing the level of circulating fatty acids.[27] Species in the genus *Eubacterium* were shown to reduce the amount of cholesterol available in the intestine by conversion of cholesterol to coprostanol.[28] However, previous studies did not find a sustained change in stool or serum butyrate despite engraftment of butyrate-producing bacteria.[8 10] The latter suggests that additional measures such as an increase in dietary fibre may be required for the engrafted microorganisms to exert their functions. In addition, we found that combination of FMT and lifestyle intervention has an additional beneficial effect on reducing liver stiffness. A previous study observed no significant changes in hepatic proton density fat fraction in patients with non-alcoholic fatty liver disease following FMT.[29] Overall, our findings indicate that combining FMT with lifestyle intervention has significant beneficial effects over FMT alone in obese patients with T2DM.

We did not observe significant weight loss after FMT. An increase in butyrate-producing bacteria enhances recipients' microbiota to digest fibre into short-chain fatty acids (SCFAs). SCFAs may promote weight loss through the release of GLP-1 that promotes satiety and the activation of brown adipose tissue via gut–brain neural circuit.[27 30] However, a recent randomised trial showed no significant increase of GLP-1 following FMT.[10] An alternative hypothesis is that response to FMT is driven by recipient's baseline microbial profile. A low gut microbiota diversity has been shown to predict metabolic response to FMT.[8] Indeed, two obese subjects in our study achieved over 10% weight loss while others showed no meaningful weight reduction following FMT from the same pool of lean donors. It is possible that only a subset of individuals may respond to modulation of the gut microbiota.

The strengths of this study include a rigorous randomised study design with sham controls and inclusion of lifestyle intervention that allows us, for the first time, to compare microbiota transfer through FMT and lifestyle intervention in parallel. We used metagenomic sequencing to characterise both donor and recipient gut bacterial communities. Using mixed samples from multiple donors had increased the extent and duration of donor engraftment and minimised the chance of receiving unfavourable donor microbiota.

Our study had limitations. First, it has been suggested that metabolic and microbiome changes are short-lived. Additional dose-finding studies will be needed to define the optimal dosing and duration of FMT. Second, the recipients' compliance to lifestyle intervention was suboptimal. However, our results highlight the practical difficulties of intensive dietary intervention. Third, our trial needs to be repeated in other geographical locations since microbiome composition is affected by ethnicity and diet.[31 32] Lastly, plasma glucose and insulin alone are not reliable measurements to determine glucose metabolism in humans. Dynamic tests such as insulin clamp to measure insulin resistance should be used in future studies.

In conclusion, we have shown that repeated FMTs led to a significant and sustained increase in microbiota engraftment in obese patients with T2DM. Combining FMT with lifestyle intervention further improves recipients' microbiota profile and metabolic parameters. Further studies are needed to optimise donor selection to improve clinical outcomes with FMT in patients with obesity and metabolic diseases.

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Contributors SCN and FKLC contributed to the study design, direction, guidance and manuscript writing; ZX and KY performed microbiome analysis and drafting of manuscript; JWYM and AYLL contributed to analysis of clinical data and manuscript preparation; CHH performed lifestyle intervention; TZ, QL, PC and WT contributed to study design; YKT provided statistical input and analysis; VW-SW performed fibroscan; SW, AP-SK, RM, EC, KC and JYLC contributed to subject recruitment, sample collection and processing; SKHW, LL and RNL performed FMT/sham infusion procedures.

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Competing interests SCN has served as advisory board member for Pfizer, Ferring, Janssen, Abbvie and speaker for Ferring, Tillotts, Menarini, Janssen, Abbvie and Takeda. She has received research grants from Olympus, Ferring and Abbvie. FKLC has served as advisor and Lecture Speaker for Eisai Co. Ltd, AstraZeneca, Pfizer Inc, Takeda Pharmaceutical Co and Takeda (China) Holdings Co. Ltd. VW-SW has served as a consultant or advisory board member for 3V-BIO, AbbVie, Allergan, Boehringer Ingelheim, Center for Outcomes Research in Liver Diseases, Echosens, Gilead Sciences, Hanmi Pharmaceutical, Intercept, Merck, Novartis, Novo Nordisk, Perspectum Diagnostics, Pfizer, ProSciento, Sagimet Biosciences, TARGET PharmaSolutions and Terns; and a speaker for AbbVie, Bristol-Myers Squibb, Echosens and Gilead Sciences. He has received a grant from Gilead Sciences for fatty liver research. ZX: none to declare. JWYM: none to declare. KY: none to declare. QL: none to declare. TZ: none to declare. WT: none to declare. LL: none to declare. RNL: none to declare. SW: none to declare. YKT: none to declare. AYLL: none to declare. KC: none to declare. JYLC: none to declare. AP-SK: none to declare. RM: none to declare. EC: none to declare. SKHW: none to declare. CHH: none to declare. PC: none to declare.

Patient consent for publication Not required.

Ethics approval The Joint Chinese University of Hong Kong New Territories East Cluster Clinical Research Ethics Committee approved this study (2016.136-T).

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REFERENCES

1 Wu T, Gao X, Chen M, *et al*. Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis. *Obes Rev* 2009;10:313–23.
2 Ley RE, Bäckhed F, Turnbaugh P, *et al*. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* 2005;102:11070–5.
3 Le Chatelier E, Nielsen T, Qin J, *et al*. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013;500:541–6.
4 Turnbaugh PJ, Ley RE, Mahowald MA, *et al*. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–31.
5 Qin J, Li Y, Cai Z, *et al*. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012;490:55–60.
6 Turnbaugh PJ, Hamady M, Yatsunenko T, *et al*. A core gut microbiome in obese and lean twins. *Nature* 2009;457:480–4.
7 Vrieze A, Van Nood E, Holleman F, *et al*. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012;143:913–6.
8 Kootte RS, Levin E, Salojärvi J, *et al*. Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab* 2017;26:611–9.
9 Yu EW, Gao L, Stastka P, *et al*. Fecal microbiota transplantation for the improvement of metabolism in obesity: the FMT-TRIM double-blind placebo-controlled pilot trial. *PLoS Med* 2020;17:e1003051.

10 Allegretti JR, Kassam Z, Mullish BH, *et al*. Effects of fecal microbiota transplantation with oral capsules in obese patients. *Clin Gastroenterol Hepatol* 2020;18:855–63.
11 de Groot P, Nikolic T, Pellegrini S, *et al*. Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut* 2021;70:92–105.
12 Ridaura VK, Faith JJ, Rey FE, *et al*. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013;341:1241214.
13 Rinott E, Youngster I, Yaskolka Meir A, *et al*. Effects of diet-modulated autologous fecal microbiota transplantation on weight regain. *Gastroenterology* 2021;160:158–73.
14 WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004;363:157–63.
15 Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for illumina sequence data. *Bioinformatics* 2014;30:2114–20.
16 Truong DT, Franzosa EA, Tickle TL, *et al*. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nat Methods* 2015;12:902–3.
17 Franzosa EA, McIver LJ, Rahnavard G, *et al*. Species-level functional profiling of metagenomes and metatranscriptomes. *Nat Methods* 2018;15:962–8.
18 Wickham H, Averick M, Bryan J, *et al*. Welcome to the Tidyverse. *J Open Source Softw* 2019;4:1686.
19 Wickham H. ggpubr: 'ggplot2' Based Publication Ready Plots gpubr (V.0.4.0). Available: https://rpkgs.datanovia.com/ggpubr/
20 McMurdie PJ, Holmes S. Phyloseq: a Bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. *Pac Symp Biocomput* 2012:235–46.
21 Emiola A, Oh J. High throughput in situ metagenomic measurement of bacterial replication at ultra-low sequencing coverage. *Nat Commun* 2018;9:4956.
22 Chen W, Gong L, Guo Z, *et al*. A novel integrated method for large-scale detection, identification, and quantification of widely targeted metabolites: application in the study of rice metabolomics. *Mol Plant* 2013;6:1769–80.
23 Li SS, Zhu A, Benes V, *et al*. Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Science* 2016;352:586–9.
24 Segata N, Izard J, Waldron L, *et al*. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;12:R60.
25 Paramsothy S, Kamm MA, Kaakoush NO, *et al*. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. *The Lancet* 2017;389:1218–28.
26 Martinez-Guryn K, Hubert N, Frazier K, *et al*. Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. *Cell Host Microbe* 2018;23:458–69.
27 Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 2015;11:577–91.
28 Kenny DJ, Plichta DR, Shungin D, *et al*. Cholesterol metabolism by uncultured human gut bacteria influences host cholesterol level. *Cell Host Microbe* 2020;28:245–57.
29 Craven L, Rahman A, Nair Parvathy S, *et al*. Allogenic fecal microbiota transplantation in patients with nonalcoholic fatty liver disease improves abnormal small intestinal permeability: a randomized control trial. *Am J Gastroenterol* 2020;115:1055–65.
30 Li Z, Yi C-X, Katiraei S, *et al*. Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut* 2018;67:1269–79.
31 Deschasaux M, Bouter KE, Prodan A, *et al*. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nat Med* 2018;24:1526–31.
32 Yatsunenko T, Rey FE, Manary MJ, *et al*. Human gut microbiome viewed across age and geography. *Nature* 2012;486:222–7.

Appendix

Inclusion criteria

1 Age 18-70; and

2 BMI ≥28 kg/m2 and < 45 kg/m2; and

3 A diagnosis of Type 2 diabetes mellitus for ≥3 months; and

4 Written informed consent obtained

Exclusion criteria

1 Current pregnancy

2 Use of any weight loss medications in the preceding 1 year

3 Known history or concomitant significant gastrointestinal disorders (including Inflammatory Bowel Disease, current colorectal cancer, current gastrointestinal infection)

4 Known history or concomitant significant food allergies

5 Immunosuppressed subjects

6 Known history of severe organ failure (including decompensated cirrhosis), inflammatory bowel disease, kidney failure, epilepsy, acquired immunodeficiency syndrome

7 Current active sepsis

8 Active malignant disease in recent 2 years

9 Known contraindications to oesophago-gastro-duodenoscopy (OGD)

10 Use of probiotic or antibiotics in recent 3 months

11 On Sodium-glucose co-transporter-2 inhibitors or Glucagon-like peptide-1 receptor agonists at randomisation

12 On Proton-pump inhibitor at randomisation

Prohibited medications

No antibiotics, probiotic or prebiotic preparations, Sodium-glucose co-transporter-2 (SGLT2) inhibitors, Glucagon-like peptide-1 (GLP-1) receptor agonists or Proton-pump inhibitor (PPI) permitted during the study.

Subjects with intake of prohibited medication during study period will remain in study and outcome will be assessed. The reported intake of prohibited medication will be recorded and documented.

Donor selection

Stool donated from donors (BMI < 23kg/m^2) of two independent studies [(CRE. 2016.033) Study title: Development of donor screening and systematic stool bank for Faecal Microbiota Transplantation (Stool bank study)] or [(CRE. 2016.707) Study title: Stool Biobank of the Faculty of Medicine, The Chinese University of Hong Kong] will be processed and used for FMT infusion. In the aforementioned studies, volunteers from general population including spouses or partners, first-degree relatives, other relatives, friends and others who are known or unknown to the potential subjects that met eligibility criteria will be invited for screening laboratory test. A series of laboratory tests for infectious diseases and interviews will be done. Stool from the eligible donors will be used in this study. Subjects may receive stools from single or multiple donors whose identity may not be made available to the subjects.

Lifestyle intervention

The lifestyle intervention principally targeted at body weight loss via decreasing calorie intake while enhancing energy expenditure. In addition to diet, our protocol emphasised lifestyle and behavioural changes to facilitate sustainable weight loss. During the first consultation session at baseline, a 1-hour comprehensive assessment regarding medical history, dietary and lifestyle habits and behaviours, knowledge on diet-disease relationships, motivation of lifestyle changes was conducted by a designated dietitian to discuss the goals of treatment and provided individualised dietary and lifestyle advice. During subsequent 20-minute follow-up sessions, the dietitian reviewed the compliance of dietary and lifestyle and provided further recommendation where appropriate.

A personalised diet plan was given to each subject at the first session, based on the recommendations of the American Dietetic Association.[1] The diet plan was nutritionally balanced with emphasis on adequate fruits and vegetables, moderate carbohydrate, low fat and glycaemic index food options. A set of 2 booklets containing the diet plan, food portion exchanges, general tips for eating out was given to each subject for reference.

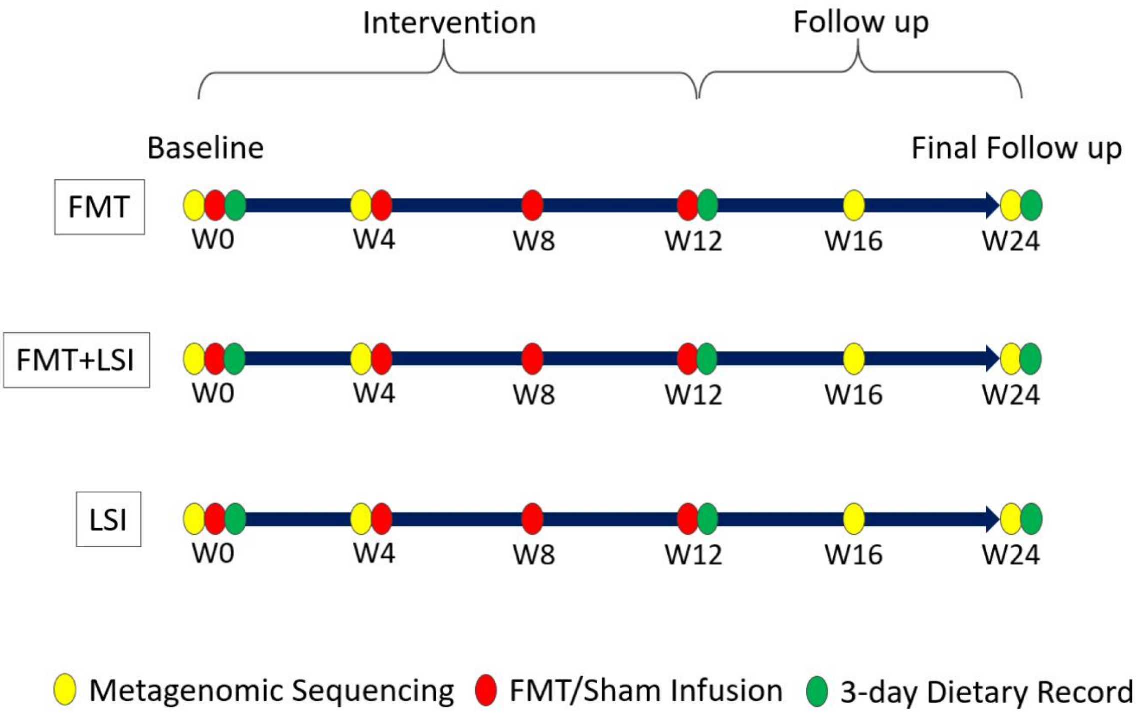
Other than practical dietary and lifestyle advice, multiple approaches were employed to foster sustainable healthier behavioural changes during the intervention period. Subjects were advised to record their diet and physical activity daily prior to each review session to develop self-monitoring behaviour, while negotiated strategies to deal with ‘at-risk’ scenarios such as stress situations, travelling, festive and party eating. Recipes with simple instructions were also given to subjects where appropriate to encourage healthy cooking.

The adherence of lifestyle intervention was assessed by the percentage attendance of dietitian sessions during the 12-week treatment period and the weekly dietary and lifestyle record at each follow-up session.

Reference

1. Wheeler ML, Franz M, Barrier P, Holler H, Cronmiller N, Delahanty LM. Macronutrient and energy database for the 1995 Exchange Lists for Meal Planning: a rationale for clinical practice decisions. J Am Diet Assoc 1996; 96(11): 1167-71.

Supplementary figure 1: Study flow



Supplementary table 1. Summary of stool donors' characteristics

Total (n=6)	Donor characteristics
Male, n (%)	4 (66.7)
Age, years (P25-P75)	29 (24-41)
Smoker/ Ex-smoker, n (%)	0 (0%)
Alcoholic drinker/ Ex-drinker, n (%)	0 (0%)
Body weight, kg (P25-P75)	54.5 (53.0-64.3)
BMI, kg/m2 (P25-P75)	20.1 (19.3-21.6)

Supplementary Table 2. Differential taxa between lean donors and recipient baseline microbiota identified by LEfSe (LDA>2, adjusted p<0.05).

Phylum	**genera/species**	**Log10 Relative Abundance**	**group**	**LDA**	**Adjusted p value**
Firmicutes	Faecalibacterium_prausnitzii	4.974	donor	4.494	0.007
Firmicutes	Faecalibacterium	4.974	donor	4.494	0.007
Firmicutes	Roseburia_inulinivorans	4.459	donor	4.040	0.006
Firmicutes	Roseburia	4.499	donor	4.034	0.006
Firmicutes	Veillonella	3.826	donor	3.503	0.025
Bacteroidetes	Bacteroides_fragilis	3.879	donor	3.398	0.028
Proteobacteria	Burkholderiales_bacterium_1_1_47	3.613	donor	3.347	0.039
Proteobacteria	Haemophilus	3.507	donor	3.288	0.000
Proteobacteria	Haemophilus_parainfluenzae	3.486	donor	3.267	0.000
Proteobacteria	Shigella_sonnei	2.595	donor	3.058	0.045
Bacteroidetes	Alistipes_sp_HGB5	2.377	donor	2.896	0.002
Firmicutes	Clostridium_celatum	1.259	donor	2.894	0.000
Proteobacteria	Actinobacillus	1.096	donor	2.860	0.045
Proteobacteria	Haemophilus_pittmaniae	1.422	donor	2.842	0.002
Bacteroidetes	Bacteroides_sp_2_1_22	3.070	donor	2.775	0.045
Firmicutes	Anaerostipes_hadrus	3.250	donor	2.741	0.019
Firmicutes	Leuconostoc_kimchii	1.206	donor	2.663	0.002
Proteobacteria	Haemophilus_sputorum	2.090	donor	2.645	0.001
Firmicutes	Veillonella_parvula	3.080	donor	2.631	0.016
Firmicutes	Clostridium_bartlettii	2.944	donor	2.419	0.002
Proteobacteria	Citrobacter	2.731	donor	2.375	0.010
Firmicutes	Anaerococcus_lactolyticus	0.181	donor	2.323	0.045
Firmicutes	Subdoligranulum	4.639	recipient	4.210	0.035
Firmicutes	Lactobacillus	4.061	recipient	3.770	0.041
Firmicutes	Streptococcus_lutetiensis	1.439	recipient	2.021	0.019

Supplementary Table 3. Clinical parameters by treatment groups at baseline and at week 24

	FMT with LSI			**FMT**			**Sham with LSI**		
Parameters	**Baseline**	**Week 24**	**p-value**	**Baseline**	**Week 24**	**p-value**	**Baseline**	**Week 24**	**p-value**
Fasting glucose, mmol/l	7.111± 2.556	7.605± 2.021	0.481	6.558± 1.988	7.632± 2.805	0.114	6.068± 0.874	7.263± 2.271	0.047
HbA1c, %	7.663± 1.368	7.453± 1.301	0.443	7.568± 1.391	7.526± 1.511	0.868	7.612± 1.236	6.982± 1.373	0.074
Fasting insulin, mIU/l	31.921± 39.357	26.005± 21.082	0.283	49.839± 79.330	48.256± 97.436	0.922	21.094± 9.493	20.728± 8.825	0.798
HOMA-IR	3.215± 2.370	3.135± 2.193	0.770	5.465± 7.805	5.287± 8.728	0.869	2.759± 1.163	2.883± 1.337	0.576
Triglycerides, mmol/l	1.684± 0.971	1.463± 0.849	0.314	2.028± 1.201	1.839± 1.211	0.278	1.974± 1.058	1.726± 0.608	0.234
Total Cholesterol, mmol/l	4.363± 0.758	3.953± 0.788	0.050	4.339± 0.654	4.311± 0.693	0.837	3.958± 0.620	3.963± 0.690	0.955
HDL cholesterol, mmol/l	1.353± 0.204	1.305± 0.239	0.207	1.339± 0.331	1.278± 0.298	0.158	1.232± 0.320	1.242± 0.280	0.821
LDL cholesterol, mmol/l	2.342± 0.720	1.989± 0.645	0.034	2.129± 0.440	2.306± 0.517	0.261	1.811± 0.536	1.937± 0.605	0.308
Liver Stiffness, kPa	6.900± 3.895	6.447± 3.056	0.461	7.963± 4.232	6.647± 2.453	0.242	8.928± 5.346	7.744± 3.715	0.302
Liver CAP, dB/m	330.530± 43.361	299.790± 67.265	0.006	330.000± 52.748	327.680± 40.556	0.801	332.056± 53.877	318.222± 42.445	0.265

Data expressed as mean±SD. Significance was calculated by paired sample t test.

Supplementary table 4. Adverse events by treatment groups

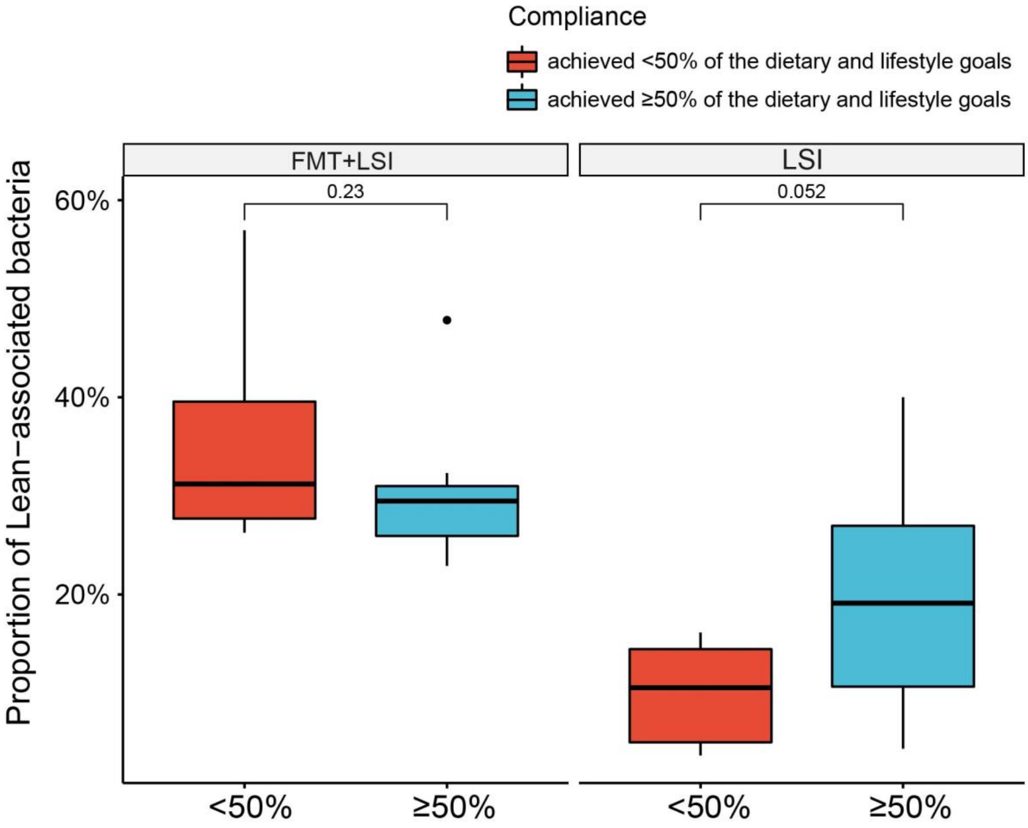
Adverse events	**FMT with lifestyle intervention (n=21)**	**FMT (n=20)**	**Sham with lifestyle intervention (n=20)**
Gastrointestinal disorders	7 (33.3%)	8 (40%)	7 (35%)
Abdominal pain	/	/	2
Belching	1	/	/
Constipation	1	1	/
Diarrhea	1	1	/
Duodenum ulcer	/	/	1
Esophageal ulcer	1	/	/
Esophagitis	1	1	/
Gastric erosion	1	1	/
Gastric reflux	/	/	1
Gastritis	/	2	2
Toothache	/	2	1
Nausea and vomiting	1	/	/
Musculoskeletal disorders	2 (9.5%)	0	4 (20%)
Eye disorders[^]	/	2 (10%)	/
Cardiac disorders[#]	/	1 (5%)	2 (10%)
Others[*]	8 (38%)	8 (40%)	13 (65%)

Cardiac disorders included abnormal ECG, paroxysmal atrial flutter and sinus tachycardia.

[^] Eye disorders included floaters and blurred vision.

[*] Others included flu, hypoglycemia, psoriasis, skin rash, tinnitus, tonsillitis, slip and fall.

Supplementary figure 2: Subgroup analysis of the percentage of lean-associated microbiota between subjects with different compliance to lifestyle modification in FMT plus LSI arm and sham plus LSI arm.



Engraftment of Species in Each Recipient

Species	engraftment_rate*	FDM001	FDM002	FDM004	FDM006	FDM008	FDM009	FDM010
s__Collinsella_takaei	59.5%	Y		Y		Y		Y
s__Roseburia_hominis	51.4%	Y	Y	Y	Y			
s__Bacteroidales_bacterium_ph8	48.6%	Y			Y	Y		Y
s__Lachnospiraceae_bacterium_3_1_46FAA	48.6%		Y	Y			Y	Y
s__Aerostipes_hadrus	48.6%			Y		Y		Y
s__Rothia_mucilaginosa	48.6%			Y	Y	Y		Y
s__Eubacterium_ramulus	43.2%	Y	Y	Y			Y	
s__Eubacterium_ventriosum	43.2%	Y		Y				
s__Blautia_hydrogenotrophica	43.2%			Y	Y	Y		Y
s__Lactococcus_garvieae	43.2%			Y		Y	Y	Y
s__Barnesiella_intestinihominis	40.5%	Y						
s__Eubacterium_cylindroides	40.5%			Y	Y	Y	Y	
s__Collinsella_intestilis	40.5%							
s__Eubacterium_eligens	37.8%	Y					Y	
s__Prevotella_copri	37.8%		Y				Y	Y
s__Streptococcus_infantis	37.8%		Y					
s__Streptococcus_thermophilus	37.8%		Y			Y	Y	
s__Bacteroides_eggerthii	37.8%			Y		Y	Y	
s__Bacteroides_caccae	37.8%						Y	Y
s__Sutterella_wadsworthensis	37.8%							
g__Paraprevotella_unclassified	35.1%	Y					Y	Y
s__Alistipes_onderdonkii	35.1%	Y					Y	
s__Bifidobacterium_bifidum	35.1%	Y					Y	Y
s__Desulfovibrio_piger	35.1%	Y	Y				Y	Y
s__Eubacterium_rectale	35.1%	Y					Y	
s__Slackia_piriformis	35.1%	Y	Y					Y
s__Bacteroides_dorei	35.1%		Y	Y				Y
s__Actinomyces_graevenitzii	32.4%	Y		Y	Y	Y		
s__Actinomyces_odontolyticus	32.4%	Y		Y	Y		Y	
s__Coprococcus_sp_ART55_1	32.4%	Y					Y	Y
s__Enterococcus_faecalis	32.4%	Y	Y		Y	Y	Y	Y
s__Paraprevotella_clara	32.4%	Y						Y
s__Streptococcus_vestibularis	32.4%	Y			Y			
s__Alistipes_shahii	32.4%		Y				Y	
s__Gordonibacter_pamelaeae	32.4%			Y				Y
s__Ruminococcus_bromii	32.4%				Y	Y		Y
s__Haemophilus_parainfluenzae	32.4%					Y		
s__Streptococcus_anginosus	32.4%					Y		

* Engraftment refers to a species that were present in donors, absent at baseline, and present in at least one of post-FMT samples in each recipient

Only species engrafted in over 30% of recipients were shown

Engraftment of Species in Each Recipient

Species	FDM012	FDM013	FDM014	FDM016	FDM017	FDM019	FDM020	FDM022	FDM024
s__Collinsella_takaei		Y			Y	Y	Y	Y	Y
s__Roseburia_hominis		Y	Y	Y	Y		Y	Y	
s__Bacteroidales_bacterium_ph8			Y	Y				Y	
s__Lachnospiraceae_bacterium_3_1_46FAA				Y			Y	Y	Y
s__Aerostipes_hadrus					Y	Y			Y
s__Rothia_mucilaginosa	Y	Y	Y	Y	Y		Y		
s__Eubacterium_ramulus		Y				Y		Y	Y
s__Eubacterium_ventriosum					Y		Y	Y	Y
s__Blautia_hydrogenotrophica		Y		Y	Y			Y	Y
s__Lactococcus_garvieae			Y	Y		Y			
s__Barnesiella_intestinihominis	Y	Y							
s__Eubacterium_cylindroides		Y							Y
s__Collinsella_intestilis						Y			Y
s__Eubacterium_eligens	Y		Y	Y				Y	
s__Prevotella_copri	Y								
s__Streptococcus_infantis		Y	Y	Y	Y				
s__Streptococcus_thermophilus			Y	Y			Y		
s__Bacteroides_eggerthii		Y	Y	Y			Y		
s__Bacteroides_caccae						Y	Y	Y	Y
s__Sutterella_wadsworthensis									
g__Paraprevotella_unclassified	Y	Y							
s__Alistipes_onderdonkii									Y
s__Bifidobacterium_bifidum	Y								Y
s__Desulfovibrio_piger	Y								
s__Eubacterium_rectale		Y			Y		Y	Y	Y
s__Slackia_piriformis	Y	Y							
s__Bacteroides_dorei			Y				Y		Y
s__Actinomyces_graevenitzii					Y				
s__Actinomyces_odontolyticus	Y		Y	Y				Y	
s__Coprococcus_sp_ART55_1	Y	Y							
s__Enterococcus_faecalis	Y			Y					
s__Paraprevotella_clara	Y	Y							
s__Streptococcus_vestibularis	Y				Y	Y			Y
s__Alistipes_shahii									Y
s__Gordonibacter_pamelaeae									Y
s__Ruminococcus_bromii					Y			Y	Y
s__Haemophilus_parainfluenzae					Y			Y	
s__Streptococcus_anginosus	Y						Y		

Engraftment of Species in Each Recipient

Species	FDM025	FDM026	FDM028	FDM030	FDM031	FDM034	FDM035	FDM040	FDM041
s__Collinsella_takaei				Y	Y	Y	Y	Y	
s__Roseburia_hominis	Y	Y					Y	Y	
s__Bacteroidales_bacterium_ph8	Y	Y	Y			Y	Y	Y	
s__Lachnospiraceae_bacterium_3_1_46FAA					Y	Y	Y	Y	Y
s__Aerostipes_hadrus	Y	Y	Y		Y		Y	Y	Y
s__Rothia_mucilaginosa	Y						Y	Y	
s__Eubacterium_ramulus							Y	Y	Y
s__Eubacterium_ventriosum	Y						Y	Y	
s__Blautia_hydrogenotrophica	Y					Y			
s__Lactococcus_garvieae			Y				Y		
s__Barnesiella_intestinihominis	Y		Y				Y	Y	
s__Eubacterium_cylindroides	Y			Y			Y	Y	Y
s__Collinsella_intestilis		Y		Y		Y			
s__Eubacterium_eligens	Y				Y				Y
s__Prevotella_copri									
s__Streptococcus_infantis	Y		Y		Y			Y	
s__Streptococcus_thermophilus		Y	Y				Y	Y	
s__Bacteroides_eggerthii				Y	Y		Y		
s__Bacteroides_caccae							Y	Y	
s__Sutterella_wadsworthensis	Y	Y		Y			Y	Y	
g__Paraprevotella_unclassified									Y
s__Alistipes_onderdonkii	Y	Y	Y				Y	Y	
s__Bifidobacterium_bifidum							Y		
s__Desulfovibrio_piger									
s__Eubacterium_rectale		Y					Y	Y	Y
s__Slackia_piriformis									
s__Bacteroides_dorei	Y						Y		
s__Actinomyces_graevenitzii			Y		Y		Y		
s__Actinomyces_odontolyticus					Y				
s__Coprococcus_sp_ART55_1									
s__Enterococcus_faecalis		Y		Y			Y		
s__Paraprevotella_clara									Y
s__Streptococcus_vestibularis		Y	Y						
s__Alistipes_shahii	Y						Y	Y	
s__Gordonibacter_pamelaeae	Y		Y	Y			Y		
s__Ruminococcus_bromii		Y			Y	Y			
s__Haemophilus_parainfluenzae	Y	Y	Y			Y			
s__Streptococcus_anginosus		Y	Y			Y	Y	Y	

Engraftment of Species in Each Recipient

Species	FDM042	FDM043	FDM046	FDM047	FDM048	FDM049	FDM050	FDM051	FDM055
s__Collinsella_takaei	Y		Y		Y	Y		Y	Y
s__Roseburia_hominis			Y				Y	Y	Y
s__Bacteroidales_bacterium_ph8					Y	Y	Y	Y	Y
s__Lachnospiraceae_bacterium_3_1_46FAA	Y		Y		Y				Y
s__Aerostipes_hadrus	Y	Y							Y
s__Rothia_mucilaginosa						Y	Y		
s__Eubacterium_ramulus	Y			Y					Y
s__Eubacterium_ventriosum	Y	Y		Y	Y		Y	Y	Y
s__Blautia_hydrogenotrophica	Y	Y					Y	Y	
s__Lactococcus_garvieae					Y		Y	Y	Y
s__Barnesiella_intestinihominis	Y		Y	Y	Y	Y	Y	Y	
s__Eubacterium_cylindroides			Y		Y	Y		Y	
s__Collinsella_intestilis	Y		Y	Y	Y	Y	Y	Y	Y
s__Eubacterium_eligens		Y	Y				Y		
s__Prevotella_copri		Y		Y	Y	Y	Y	Y	Y
s__Streptococcus_infantis						Y	Y		
s__Streptococcus_thermophilus	Y					Y			
s__Bacteroides_eggerthii					Y	Y			Y
s__Bacteroides_caccae	Y	Y		Y	Y	Y	Y		
s__Sutterella_wadsworthensis	Y	Y	Y	Y	Y		Y	Y	
g__Paraprevotella_unclassified		Y		Y	Y	Y	Y	Y	Y
s__Alistipes_onderdonkii		Y	Y		Y	Y		Y	
s__Bifidobacterium_bifidum	Y			Y		Y		Y	Y
s__Desulfovibrio_piger		Y		Y	Y	Y	Y	Y	Y
s__Eubacterium_rectale				Y				Y	
s__Slackia_piriformis		Y		Y	Y	Y	Y	Y	Y
s__Bacteroides_dorei	Y				Y			Y	Y
s__Actinomyces_graevenitzii			Y	Y					
s__Actinomyces_odontolyticus					Y			Y	
s__Coprococcus_sp_ART55_1		Y		Y		Y	Y	Y	
s__Enterococcus_faecalis							Y		
s__Paraprevotella_clara		Y		Y	Y	Y	Y	Y	Y
s__Streptococcus_vestibularis			Y						Y
s__Alistipes_shahii	Y		Y	Y		Y		Y	Y
s__Gordonibacter_pamelaeae	Y		Y		Y	Y			
s__Ruminococcus_bromii	Y				Y				
s__Haemophilus_parainfluenzae		Y	Y						Y
s__Streptococcus_anginosus	Y	Y							

Engraftment of Species in Each Recipient

Species	FDM059	FDM060	FDM061
s__Collinsella_takaei		Y	
s__Roseburia_hominis	Y		
s__Bacteroidales_bacterium_ph8			
s__Lachnospiraceae_bacterium_3_1_46FAA			Y
s__Aerostipes_hadrus	Y	Y	
s__Rothia_mucilaginosa	Y	Y	Y
s__Eubacterium_ramulus		Y	Y
s__Eubacterium_ventriosum			
s__Blautia_hydrogenotrophica	Y		
s__Lactococcus_garvieae	Y	Y	Y
s__Barnesiella_intestinihominis		Y	
s__Eubacterium_cylindroides			
s__Collinsella_intestilis	Y	Y	
s__Eubacterium_eligens		Y	Y
s__Prevotella_copri	Y	Y	Y
s__Streptococcus_infantis	Y	Y	Y
s__Streptococcus_thermophilus	Y		Y
s__Bacteroides_eggerthii	Y		
s__Bacteroides_caccae			
s__Sutterella_wadsworthensis	Y	Y	
g__Paraprevotella_unclassified			
s__Alistipes_onderdonkii			
s__Bifidobacterium_bifidum	Y	Y	
s__Desulfovibrio_piger		Y	
s__Eubacterium_rectale			
s__Slackia_piriformis		Y	
s__Bacteroides_dorei	Y		
s__Actinomyces_graevenitzii		Y	Y
s__Actinomyces_odontolyticus			Y
s__Coprococcus_sp_ART55_1		Y	Y
s__Enterococcus_faecalis			
s__Paraprevotella_clara			
s__Streptococcus_vestibularis	Y		Y
s__Alistipes_shahii			
s__Gordonibacter_pamelaeae			Y
s__Ruminococcus_bromii			Y
s__Haemophilus_parainfluenzae	Y	Y	
s__Streptococcus_anginosus		Y	Y

Detailed Breakdown of Four Groups

Species	FDM001W16	FDM001W24	FDM001W4	FDM002W16	FDM002W24	FDM002W4
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Eubacterium_rectale	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Bifidobacterium_longum	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Alistipes_putredinis	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Bacteroides_caccae	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Bacteroides_massiliensis	absent	absent	absent	co-existed	co-existed	co-existed
Bacteroides_stercoris	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Clostridium_leptum	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Bacteroides_uniformis	co-existed	co-existed	co-existed	absent	co-existed	absent
Escherichia_unclassified	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Streptococcus_parasanguinis	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_dorei	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Anaerostipes_hadrus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_adolescentis	lean-associated	lean-associated	lean-associated	absent	absent	absent
Eubacterium_ventriosum	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Barnesiella_intestinihominis	lean-associated	lean-associated	lean-associated	absent	absent	absent
Lachnospiraceae_bacterium_3_1_46FAA	absent	absent	absent	lean-associated	absent	absent
Ruminococcus_gnavus	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Parabacteroides_distasonis	absent	absent	absent	co-existed	co-existed	co-existed
Megasphaera_unclassified	absent	lean-associated	absent	co-existed	absent	co-existed
Ruminococcus_bromii	co-existed	co-existed	co-existed	absent	absent	absent
Acidaminococcus_unclassified	absent	absent	absent	absent	absent	absent
Adlercreutzia_equolifaciens	co-existed	absent	co-existed	co-existed	absent	absent
Streptococcus_australis	absent	absent	absent	lean-associated	absent	lean-associated
Bacteroides_ovatus	co-existed	absent	co-existed	absent	co-existed	co-existed
Blautia_hydrogenotrophica	absent	absent	absent	absent	absent	absent
Roseburia_hominis	lean-associated	lean-associated	lean-associated	absent	lean-associated	absent
Eubacterium_cylindroides	co-existed	co-existed	co-existed	absent	absent	absent
Clostridium_bolteae	absent	lean-associated	lean-associated	co-existed	co-existed	co-existed
Actinomyces_odontolyticus	absent	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bacteroides_thetaiotaomicron	co-existed	co-existed	co-existed	absent	lean-associated	lean-associated
Alistipes_shahii	co-existed	co-existed	absent	absent	lean-associated	absent
Streptococcus_vestibularis	lean-associated	absent	absent	co-existed	co-existed	co-existed
Holdemania_filiformis	absent	co-existed	absent	absent	lean-associated	absent
Eggerthella_unclassified	absent	absent	absent	co-existed	co-existed	co-existed
Klebsiella_pneumoniae	co-existed	co-existed	absent	lean-associated	absent	lean-associated
Bacteroidales_bacterium_ph8	absent	lean-associated	absent	absent	absent	absent
Prevotella_copri	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Streptococcus_anginosus	absent	absent	absent	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	absent	absent	absent	co-existed	co-existed	co-existed
Bilophila_wadsworthia	absent	absent	lean-associated	absent	absent	absent
Desulfovibrio_piger	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated

Detailed Breakdown of Four Groups

Species	FDM004W16	FDM004W24	FDM004W4	FDM006W16	FDM006W24	FDM006W4
Collinsella_aerofaciens	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Dorea_formicigenerans	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Dorea_longicatena	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Parabacteroides_merdae	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_5_1_63FAA	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	lean-associated	lean-associated	lean-associated	absent	co-existed	co-existed
Eubacterium_ramulus	lean-associated	lean-associated	lean-associated	co-existed	co-existed	absent
Coprococcus_catus	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Eubacterium_rectale	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Bacteroides_caccae	absent	absent	co-existed	co-existed	co-existed	co-existed
Bacteroides_massiliensis	lean-associated	lean-associated	lean-associated	absent	co-existed	co-existed
Bacteroides_stercoris	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Coprococcus_comes	absent	absent	absent	absent	absent	absent
Clostridium_leptum	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Oscillibacter_unclassified	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bacteroides_uniformis	co-existed	absent	co-existed	co-existed	absent	absent
Escherichia_unclassified	lean-associated	lean-associated	lean-associated	absent	co-existed	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bacteroides_dorei	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Anaerostipes_hadrus	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ventriosum	lean-associated	lean-associated	lean-associated	absent	absent	absent
Barnesiella_intestinihominis	lean-associated	absent	absent	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	lean-associated	absent	absent	absent	absent
Ruminococcus_gnavus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_distasonis	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Megasphaera_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_bromii	co-existed	co-existed	co-existed	lean-associated	absent	lean-associated
Acidaminococcus_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	absent	co-existed	co-existed	absent	absent	absent
Streptococcus_australis	lean-associated	absent	lean-associated	absent	lean-associated	absent
Bacteroides_ovatus	co-existed	absent	absent	absent	absent	absent
Blautia_hydrogenotrophica	lean-associated	absent	absent	lean-associated	lean-associated	absent
Roseburia_hominis	lean-associated	absent	lean-associated	lean-associated	absent	absent
Eubacterium_cylindroides	lean-associated	lean-associated	lean-associated	lean-associated	absent	lean-associated
Clostridium_bolteae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Actinomyces_odontolyticus	lean-associated	lean-associated	lean-associated	absent	lean-associated	absent
Bacteroides_thetaiotaomicron	co-existed	absent	absent	absent	absent	absent
Alistipes_shahii	absent	absent	absent	lean-associated	absent	absent
Streptococcus_vestibularis	co-existed	co-existed	co-existed	absent	lean-associated	absent
Holdemania_filiformis	absent	absent	lean-associated	co-existed	co-existed	co-existed
Eggerthella_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Klebsiella_pneumoniae	lean-associated	absent	lean-associated	absent	absent	absent
Bacteroidales_bacterium_ph8	absent	absent	absent	lean-associated	absent	lean-associated
Prevotella_copri	absent	absent	absent	absent	absent	absent
Streptococcus_anginosus	absent	absent	absent	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	absent
Bilophila_wadsworthia	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Desulfovibrio_piger	absent	absent	absent	absent	absent	absent

Detailed Breakdown of Four Groups

Species	FDM008W16	FDM008W24	FDM008W4	FDM009W16	FDM009W24	FDM009W4
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	lean-associated	lean-associated	absent
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	absent	co-existed	absent	co-existed	co-existed	co-existed
Eubacterium_ramulus	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Coprococcus_catus	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Eubacterium_rectale	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Bacteroides_caccae	co-existed	co-existed	co-existed	lean-associated	lean-associated	absent
Bacteroides_massiliensis	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_stercoris	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Coprococcus_comes	absent	absent	absent	co-existed	co-existed	co-existed
Clostridium_leptum	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_unclassified	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	absent
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Bacteroides_dorei	co-existed	co-existed	absent	co-existed	absent	co-existed
Anaerostipes_hadrus	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ventriosum	absent	absent	absent	co-existed	co-existed	co-existed
Barnesiella_intestinihominis	co-existed	absent	absent	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_3_1_46FAA	absent	co-existed	co-existed	lean-associated	lean-associated	absent
Ruminococcus_gnavus	co-existed	co-existed	co-existed	absent	absent	lean-associated
Parabacteroides_distasonis	co-existed	absent	absent	absent	absent	co-existed
Megasphaera_unclassified	absent	absent	co-existed	co-existed	co-existed	co-existed
Ruminococcus_bromii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Acidaminococcus_unclassified	absent	absent	absent	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	absent	absent	absent	co-existed	co-existed	co-existed
Streptococcus_australis	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Bacteroides_ovatus	co-existed	co-existed	co-existed	lean-associated	absent	lean-associated
Blautia_hydrogenotrophica	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Roseburia_hominis	co-existed	absent	co-existed	co-existed	absent	co-existed
Eubacterium_cylindroides	absent	absent	lean-associated	lean-associated	absent	absent
Clostridium_bolteae	co-existed	absent	co-existed	absent	absent	absent
Actinomyces_odontolyticus	co-existed	co-existed	co-existed	absent	absent	lean-associated
Bacteroides_thetaiotaomicron	co-existed	absent	co-existed	co-existed	absent	absent
Alistipes_shahii	lean-associated	absent	absent	lean-associated	absent	lean-associated
Streptococcus_vestibularis	co-existed	absent	absent	absent	absent	absent
Holdemania_filiformis	absent	absent	lean-associated	co-existed	absent	co-existed
Eggerthella_unclassified	co-existed	co-existed	co-existed	absent	absent	absent
Klebsiella_pneumoniae	absent	absent	absent	absent	absent	absent
Bacteroidales_bacterium_ph8	lean-associated	absent	absent	co-existed	absent	co-existed
Prevotella_copri	absent	absent	absent	lean-associated	lean-associated	absent
Streptococcus_anginosus	lean-associated	lean-associated	absent	absent	absent	absent
Lachnospiraceae_bacterium_1_4_56FAA	co-existed	co-existed	co-existed	absent	absent	co-existed
Bilophila_wadsworthia	absent	absent	absent	co-existed	co-existed	co-existed
Desulfovibrio_piger	absent	absent	absent	lean-associated	lean-associated	absent

Detailed Breakdown of Four Groups

Species	FDM010W16	FDM010W24	FDM010W4	FDM012W16	FDM012W24	FDM013W16
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	absent	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Eubacterium_rectale	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Alistipes_putredinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_caccae	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bacteroides_massiliensis	co-existed	co-existed	co-existed	lean-associated	lean-associated	co-existed
Bacteroides_stercoris	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Clostridium_leptum	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_unclassified	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	absent	co-existed	co-existed	absent
Bacteroides_dorei	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Anaerostipes_hadrus	lean-associated	lean-associated	absent	absent	co-existed	absent
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Eubacterium_ventriosum	co-existed	co-existed	absent	co-existed	co-existed	co-existed
Barnesiella_intestinihominis	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Ruminococcus_gnavus	co-existed	absent	co-existed	absent	co-existed	co-existed
Parabacteroides_distasonis	absent	absent	absent	absent	absent	co-existed
Megasphaera_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_bromii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Acidaminococcus_unclassified	co-existed	co-existed	co-existed	absent	absent	lean-associated
Adlercreutzia_equolifaciens	co-existed	co-existed	co-existed	co-existed	absent	absent
Streptococcus_australis	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Bacteroides_ovatus	absent	co-existed	co-existed	co-existed	co-existed	absent
Blautia_hydrogenotrophica	lean-associated	lean-associated	lean-associated	absent	absent	lean-associated
Roseburia_hominis	co-existed	co-existed	co-existed	absent	absent	lean-associated
Eubacterium_cylindroides	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Clostridium_bolteae	absent	co-existed	co-existed	absent	absent	co-existed
Actinomyces_odontolyticus	co-existed	co-existed	co-existed	lean-associated	lean-associated	co-existed
Bacteroides_thetaiotaomicron	absent	co-existed	co-existed	absent	absent	absent
Alistipes_shahii	absent	absent	co-existed	absent	absent	co-existed
Streptococcus_vestibularis	co-existed	co-existed	co-existed	lean-associated	lean-associated	absent
Holdemania_filiformis	absent	absent	lean-associated	absent	absent	lean-associated
Eggerthella_unclassified	absent	absent	co-existed	absent	absent	absent
Klebsiella_pneumoniae	co-existed	co-existed	co-existed	lean-associated	lean-associated	co-existed
Bacteroidales_bacterium_ph8	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Prevotella_copri	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Streptococcus_anginosus	co-existed	co-existed	co-existed	lean-associated	lean-associated	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	co-existed	co-existed	co-existed	absent	co-existed	absent
Bilophila_wadsworthia	absent	co-existed	co-existed	absent	absent	co-existed
Desulfovibrio_piger	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	co-existed

Detailed Breakdown of Four Groups

Species	FDM013W24	FDM013W4	FDM014W16	FDM014W24	FDM014W4	FDM016W16
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Eubacterium_rectale	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	absent	absent	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	co-existed	co-existed	lean-associated	lean-associated	lean-associated	absent
Bacteroides_caccae	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Bacteroides_massiliensis	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_stercoris	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Clostridium_leptum	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Oscillibacter_unclassified	co-existed	co-existed	absent	co-existed	co-existed	absent
Bacteroides_uniformis	co-existed	co-existed	lean-associated	lean-associated	lean-associated	absent
Escherichia_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	absent	absent	absent	co-existed
Bifidobacterium_pseudocatenulatum	absent	absent	co-existed	co-existed	co-existed	co-existed
Bacteroides_dorei	co-existed	co-existed	absent	lean-associated	absent	co-existed
Anaerostipes_hadrus	co-existed	co-existed	absent	absent	absent	co-existed
Bifidobacterium_adolescentis	absent	absent	co-existed	co-existed	co-existed	co-existed
Eubacterium_ventriosum	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Barnesiella_intestinihominis	lean-associated	lean-associated	co-existed	co-existed	absent	absent
Lachnospiraceae_bacterium_3_1_46FAA	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Ruminococcus_gnavus	co-existed	absent	absent	absent	absent	absent
Parabacteroides_distasonis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Megasphaera_unclassified	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_bromii	absent	absent	absent	absent	absent	co-existed
Acidaminococcus_unclassified	lean-associated	lean-associated	absent	absent	absent	co-existed
Adlercreutzia_equolifaciens	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Streptococcus_australis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Bacteroides_ovatus	absent	absent	co-existed	co-existed	absent	absent
Blautia_hydrogenotrophica	lean-associated	lean-associated	co-existed	co-existed	co-existed	absent
Roseburia_hominis	lean-associated	lean-associated	absent	lean-associated	lean-associated	absent
Eubacterium_cylindroides	absent	lean-associated	absent	absent	absent	absent
Clostridium_bolteae	co-existed	co-existed	absent	absent	absent	absent
Actinomyces_odontolyticus	co-existed	co-existed	absent	lean-associated	lean-associated	lean-associated
Bacteroides_thetaiotaomicron	lean-associated	lean-associated	absent	absent	absent	absent
Alistipes_shahii	co-existed	co-existed	absent	absent	absent	absent
Streptococcus_vestibularis	absent	co-existed	absent	absent	absent	co-existed
Holdemania_filiformis	lean-associated	lean-associated	absent	absent	lean-associated	absent
Eggerthella_unclassified	lean-associated	absent	absent	absent	absent	absent
Klebsiella_pneumoniae	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Bacteroidales_bacterium_ph8	co-existed	co-existed	lean-associated	lean-associated	absent	absent
Prevotella_copri	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Streptococcus_anginosus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	co-existed	absent	absent	absent	absent	absent
Bilophila_wadsworthia	co-existed	absent	absent	absent	lean-associated	absent
Desulfovibrio_piger	co-existed	co-existed	absent	absent	absent	co-existed

Detailed Breakdown of Four Groups

Species	FDM016W24	FDM016W4	FDM017W16	FDM017W24	FDM017W4	FDM019W16
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Bilophila_unclassified	lean-associated	absent	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Eubacterium_ramulus	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Coprococcus_catus	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	co-existed	co-existed	lean-associated	lean-associated	lean-associated	lean-associated
Eubacterium_rectale	co-existed	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_caccae	co-existed	absent	co-existed	co-existed	co-existed	lean-associated
Bacteroides_massiliensis	co-existed	absent	co-existed	co-existed	co-existed	lean-associated
Bacteroides_stercoris	co-existed	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Clostridium_leptum	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Oscillibacter_unclassified	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	absent	absent	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_dorei	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Anaerostipes_hadrus	co-existed	absent	lean-associated	lean-associated	lean-associated	lean-associated
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Eubacterium_ventriosum	co-existed	co-existed	lean-associated	absent	absent	co-existed
Barnesiella_intestinihominis	co-existed	absent	co-existed	co-existed	co-existed	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	absent	co-existed	co-existed	co-existed	absent
Ruminococcus_gnavus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_distasonis	co-existed	co-existed	absent	co-existed	co-existed	absent
Megasphaera_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_bromii	co-existed	co-existed	lean-associated	lean-associated	absent	co-existed
Acidaminococcus_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	co-existed	co-existed	absent	absent	absent	co-existed
Streptococcus_australis	absent	lean-associated	lean-associated	lean-associated	lean-associated	absent
Bacteroides_ovatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Blautia_hydrogenotrophica	lean-associated	absent	lean-associated	lean-associated	absent	absent
Roseburia_hominis	lean-associated	absent	lean-associated	lean-associated	absent	absent
Eubacterium_cylindroides	co-existed	co-existed	absent	absent	absent	co-existed
Clostridium_bolteae	lean-associated	absent	co-existed	co-existed	co-existed	co-existed
Actinomyces_odontolyticus	absent	lean-associated	co-existed	co-existed	co-existed	co-existed
Bacteroides_thetaiotaomicron	absent	absent	co-existed	co-existed	co-existed	lean-associated
Alistipes_shahii	lean-associated	absent	co-existed	absent	co-existed	co-existed
Streptococcus_vestibularis	co-existed	co-existed	lean-associated	absent	absent	lean-associated
Holdemania_filiformis	co-existed	absent	absent	co-existed	absent	absent
Eggerthella_unclassified	absent	absent	absent	lean-associated	absent	absent
Klebsiella_pneumoniae	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroidales_bacterium_ph8	lean-associated	absent	co-existed	co-existed	absent	co-existed
Prevotella_copri	absent	absent	absent	absent	absent	absent
Streptococcus_anginosus	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	absent	absent	lean-associated	lean-associated	lean-associated	lean-associated
Bilophila_wadsworthia	absent	absent	absent	lean-associated	absent	absent
Desulfovibrio_piger	co-existed	absent	co-existed	co-existed	co-existed	absent

Detailed Breakdown of Four Groups

Species	FDM019W4	FDM020W16	FDM020W24	FDM020W4	FDM022W16	FDM022W24
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	lean-associated	lean-associated
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	lean-associated	lean-associated
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	lean-associated	lean-associated
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	lean-associated	lean-associated	lean-associated	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_vulgatus	lean-associated	co-existed	co-existed	co-existed	lean-associated	lean-associated
Eubacterium_ramulus	lean-associated	co-existed	co-existed	co-existed	lean-associated	lean-associated
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Eubacterium_rectale	co-existed	absent	absent	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	co-existed	co-existed	co-existed	co-existed	lean-associated	lean-associated
Bacteroides_caccae	absent	lean-associated	lean-associated	absent	lean-associated	lean-associated
Bacteroides_massiliensis	lean-associated	co-existed	co-existed	co-existed	absent	lean-associated
Bacteroides_stercoris	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Coprococcus_comes	co-existed	lean-associated	lean-associated	absent	lean-associated	lean-associated
Clostridium_leptum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	lean-associated	lean-associated
Bacteroides_uniformis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_unclassified	lean-associated	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	absent	absent	absent	co-existed	co-existed
Bacteroides_dorei	co-existed	lean-associated	lean-associated	lean-associated	absent	co-existed
Anaerostipes_hadrus	lean-associated	absent	absent	absent	co-existed	co-existed
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	co-existed	absent	lean-associated
Eubacterium_ventriosum	co-existed	lean-associated	lean-associated	lean-associated	lean-associated	absent
Barnesiella_intestinihominis	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	absent	lean-associated	lean-associated	absent	absent	lean-associated
Ruminococcus_gnavus	co-existed	absent	co-existed	absent	absent	co-existed
Parabacteroides_distasonis	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Megasphaera_unclassified	co-existed	co-existed	co-existed	co-existed	absent	absent
Ruminococcus_bromii	co-existed	absent	absent	absent	absent	lean-associated
Acidaminococcus_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	co-existed	lean-associated	absent	absent	absent	absent
Streptococcus_australis	co-existed	absent	co-existed	absent	absent	co-existed
Bacteroides_ovatus	absent	co-existed	co-existed	co-existed	absent	co-existed
Blautia_hydrogenotrophica	absent	absent	absent	absent	absent	lean-associated
Roseburia_hominis	co-existed	absent	lean-associated	absent	lean-associated	absent
Eubacterium_cylindroides	co-existed	co-existed	co-existed	co-existed	absent	absent
Clostridium_bolteae	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Actinomyces_odontolyticus	co-existed	absent	co-existed	absent	lean-associated	absent
Bacteroides_thetaiotaomicron	lean-associated	co-existed	co-existed	co-existed	absent	co-existed
Alistipes_shahii	co-existed	lean-associated	lean-associated	absent	lean-associated	lean-associated
Streptococcus_vestibularis	lean-associated	absent	absent	absent	absent	co-existed
Holdemania_filiformis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eggerthella_unclassified	absent	co-existed	co-existed	co-existed	absent	absent
Klebsiella_pneumoniae	co-existed	absent	lean-associated	lean-associated	absent	absent
Bacteroidales_bacterium_ph8	co-existed	co-existed	co-existed	co-existed	lean-associated	absent
Prevotella_copri	absent	co-existed	co-existed	co-existed	lean-associated	absent
Streptococcus_anginosus	co-existed	lean-associated	absent	absent	absent	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	lean-associated	absent	absent	absent	absent	co-existed
Bilophila_wadsworthia	lean-associated	absent	absent	absent	absent	co-existed
Desulfovibrio_piger	absent	co-existed	co-existed	co-existed	lean-associated	absent

Detailed Breakdown of Four Groups

Species	FDM024W16	FDM024W24	FDM024W4	FDM025W16	FDM025W24	FDM025W4
Collinsella_aerofaciens	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Eubacterium_ramulus	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Coprococcus_catus	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Roseburia_inulinivorans	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Eubacterium_rectale	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Bacteroides_caccae	lean-associated	absent	absent	co-existed	co-existed	co-existed
Bacteroides_massiliensis	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bacteroides_stercoris	absent	lean-associated	lean-associated	co-existed	co-existed	absent
Coprococcus_comes	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Clostridium_leptum	lean-associated	absent	lean-associated	lean-associated	lean-associated	lean-associated
Oscillibacter_unclassified	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	absent	absent	absent
Escherichia_unclassified	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bacteroides_dorei	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Anaerostipes_hadrus	lean-associated	lean-associated	absent	lean-associated	lean-associated	lean-associated
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	lean-associated	lean-associated	absent
Eubacterium_ventriosum	absent	absent	lean-associated	lean-associated	lean-associated	lean-associated
Barnesiella_intestinihominis	lean-associated	lean-associated	absent	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	lean-associated	lean-associated	co-existed	absent	absent
Ruminococcus_gnavus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_distasonis	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Megasphaera_unclassified	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Ruminococcus_bromii	lean-associated	lean-associated	lean-associated	absent	absent	absent
Acidaminococcus_unclassified	co-existed	co-existed	co-existed	lean-associated	lean-associated	absent
Adlercreutzia_equolifaciens	lean-associated	absent	absent	absent	co-existed	absent
Streptococcus_australis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Bacteroides_ovatus	absent	absent	co-existed	co-existed	co-existed	absent
Blautia_hydrogenotrophica	lean-associated	lean-associated	absent	lean-associated	lean-associated	lean-associated
Roseburia_hominis	absent	absent	absent	lean-associated	absent	absent
Eubacterium_cylindroides	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Clostridium_bolteae	absent	absent	absent	absent	absent	co-existed
Actinomyces_odontolyticus	co-existed	co-existed	co-existed	absent	absent	absent
Bacteroides_thetaiotaomicron	absent	absent	absent	co-existed	co-existed	co-existed
Alistipes_shahii	absent	lean-associated	absent	lean-associated	absent	absent
Streptococcus_vestibularis	lean-associated	absent	lean-associated	absent	absent	absent
Holdemania_filiformis	lean-associated	absent	absent	co-existed	co-existed	co-existed
Eggerthella_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Klebsiella_pneumoniae	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Bacteroidales_bacterium_ph8	absent	absent	absent	lean-associated	absent	absent
Prevotella_copri	absent	absent	absent	co-existed	co-existed	co-existed
Streptococcus_anginosus	absent	absent	absent	absent	absent	absent
Lachnospiraceae_bacterium_1_4_56FAA	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Bilophila_wadsworthia	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Desulfovibrio_piger	absent	absent	absent	absent	absent	absent

Detailed Breakdown of Four Groups

Species	FDM026W16	FDM026W24	FDM026W4	FDM028W16	FDM028W24	FDM028W4
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	absent	lean-associated	lean-associated	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	absent	co-existed	co-existed	absent	co-existed	co-existed
Coprococcus_catus	absent	absent	co-existed	absent	absent	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	co-existed	co-existed	co-existed	absent	absent	co-existed
Eubacterium_rectale	lean-associated	lean-associated	absent	absent	absent	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	absent	absent	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	co-existed	absent	co-existed	co-existed	absent	co-existed
Bacteroides_caccae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_massiliensis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_stercoris	absent	absent	co-existed	co-existed	co-existed	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Clostridium_leptum	absent	absent	lean-associated	co-existed	absent	co-existed
Oscillibacter_unclassified	absent	absent	lean-associated	co-existed	absent	co-existed
Bacteroides_uniformis	lean-associated	lean-associated	lean-associated	co-existed	co-existed	absent
Escherichia_unclassified	absent	absent	absent	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_dorei	co-existed	co-existed	co-existed	absent	absent	absent
Anaerostipes_hadrus	absent	absent	lean-associated	lean-associated	lean-associated	lean-associated
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Eubacterium_ventriosum	absent	absent	co-existed	absent	absent	co-existed
Barnesiella_intestinihominis	absent	absent	absent	lean-associated	absent	absent
Lachnospiraceae_bacterium_3_1_46FAA	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Ruminococcus_gnavus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_distasonis	absent	absent	co-existed	co-existed	co-existed	absent
Megasphaera_unclassified	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Ruminococcus_bromii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Acidaminococcus_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	absent	absent	lean-associated	absent	absent	lean-associated
Streptococcus_australis	co-existed	co-existed	co-existed	absent	absent	lean-associated
Bacteroides_ovatus	co-existed	absent	co-existed	absent	absent	absent
Blautia_hydrogenotrophica	absent	absent	absent	absent	absent	absent
Roseburia_hominis	absent	absent	lean-associated	absent	absent	absent
Eubacterium_cylindroides	absent	absent	absent	absent	absent	co-existed
Clostridium_bolteae	co-existed	absent	co-existed	lean-associated	lean-associated	absent
Actinomyces_odontolyticus	absent	co-existed	absent	absent	absent	absent
Bacteroides_thetaiotaomicron	absent	absent	co-existed	co-existed	co-existed	absent
Alistipes_shahii	absent	absent	absent	absent	absent	absent
Streptococcus_vestibularis	absent	lean-associated	absent	lean-associated	lean-associated	lean-associated
Holdemania_filiformis	absent	absent	absent	absent	co-existed	co-existed
Eggerthella_unclassified	absent	absent	lean-associated	lean-associated	lean-associated	lean-associated
Klebsiella_pneumoniae	absent	co-existed	co-existed	absent	co-existed	co-existed
Bacteroidales_bacterium_ph8	lean-associated	absent	absent	absent	lean-associated	absent
Prevotella_copri	absent	absent	absent	absent	absent	absent
Streptococcus_anginosus	absent	lean-associated	absent	absent	lean-associated	absent
Lachnospiraceae_bacterium_1_4_56FAA	lean-associated	absent	lean-associated	co-existed	absent	co-existed
Bilophila_wadsworthia	lean-associated	absent	lean-associated	co-existed	co-existed	co-existed
Desulfovibrio_piger	lean-associated	lean-associated	lean-associated	absent	absent	absent

Detailed Breakdown of Four Groups

Species	FDM030W16	FDM030W24	FDM030W4	FDM031W16	FDM031W24	FDM031W4
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Eubacterium_rectale	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_caccae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_massiliensis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Bacteroides_stercoris	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Clostridium_leptum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	absent	absent	absent	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_dorei	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Anaerostipes_hadrus	co-existed	co-existed	co-existed	lean-associated	absent	absent
Bifidobacterium_adolescentis	lean-associated	lean-associated	lean-associated	absent	absent	absent
Eubacterium_ventriosum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Barnesiella_intestinihominis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Lachnospiraceae_bacterium_3_1_46FAA	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Ruminococcus_gnavus	absent	absent	absent	absent	co-existed	co-existed
Parabacteroides_distasonis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Megasphaera_unclassified	co-existed	co-existed	co-existed	absent	absent	absent
Ruminococcus_bromii	co-existed	co-existed	co-existed	lean-associated	lean-associated	absent
Acidaminococcus_unclassified	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_australis	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Bacteroides_ovatus	absent	absent	absent	absent	absent	absent
Blautia_hydrogenotrophica	co-existed	co-existed	co-existed	absent	absent	absent
Roseburia_hominis	absent	co-existed	absent	co-existed	co-existed	co-existed
Eubacterium_cylindroides	lean-associated	lean-associated	lean-associated	absent	absent	absent
Clostridium_bolteae	absent	absent	co-existed	absent	absent	co-existed
Actinomyces_odontolyticus	absent	absent	absent	lean-associated	lean-associated	lean-associated
Bacteroides_thetaiotaomicron	co-existed	absent	absent	absent	absent	absent
Alistipes_shahii	co-existed	absent	co-existed	absent	absent	absent
Streptococcus_vestibularis	absent	absent	absent	co-existed	co-existed	co-existed
Holdemania_filiformis	co-existed	co-existed	co-existed	absent	absent	co-existed
Eggerthella_unclassified	absent	absent	lean-associated	co-existed	co-existed	co-existed
Klebsiella_pneumoniae	absent	co-existed	absent	co-existed	absent	absent
Bacteroidales_bacterium_ph8	co-existed	absent	co-existed	co-existed	co-existed	absent
Prevotella_copri	co-existed	co-existed	co-existed	absent	lean-associated	absent
Streptococcus_anginosus	absent	absent	absent	absent	absent	absent
Lachnospiraceae_bacterium_1_4_56FAA	co-existed	absent	absent	absent	absent	co-existed
Bilophila_wadsworthia	co-existed	co-existed	absent	absent	absent	absent
Desulfovibrio_piger	co-existed	co-existed	co-existed	lean-associated	lean-associated	absent

Detailed Breakdown of Four Groups

Species	FDM034W16	FDM034W24	FDM034W4	FDM035W16	FDM035W24	FDM035W4
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Parabacteroides_merdae	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	absent	absent	lean-associated	lean-associated	lean-associated
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	absent	lean-associated	lean-associated	lean-associated
Collinsella_tanakaei	lean-associated	absent	lean-associated	lean-associated	lean-associated	lean-associated
Eubacterium_rectale	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_1_1_57FAA	absent	co-existed	absent	co-existed	co-existed	co-existed
Bifidobacterium_longum	absent	absent	absent	co-existed	co-existed	co-existed
Alistipes_putredinis	absent	absent	absent	lean-associated	lean-associated	lean-associated
Bacteroides_caccae	co-existed	co-existed	co-existed	absent	lean-associated	absent
Bacteroides_massiliensis	co-existed	absent	co-existed	lean-associated	lean-associated	lean-associated
Bacteroides_stercoris	absent	absent	absent	co-existed	co-existed	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Clostridium_leptum	absent	absent	absent	lean-associated	lean-associated	lean-associated
Oscillibacter_unclassified	absent	co-existed	absent	lean-associated	lean-associated	lean-associated
Bacteroides_uniformis	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Escherichia_unclassified	absent	lean-associated	absent	absent	absent	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	absent	lean-associated	absent
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	absent	co-existed	co-existed	co-existed
Bacteroides_dorei	absent	absent	absent	absent	lean-associated	lean-associated
Anaerostipes_hadrus	absent	absent	absent	absent	absent	lean-associated
Bifidobacterium_adolescentis	absent	absent	absent	co-existed	co-existed	co-existed
Eubacterium_ventriosum	co-existed	co-existed	absent	lean-associated	lean-associated	lean-associated
Barnesiella_intestinihominis	absent	absent	absent	lean-associated	lean-associated	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	lean-associated	absent	lean-associated	lean-associated	lean-associated
Ruminococcus_gnavus	absent	absent	absent	co-existed	co-existed	co-existed
Parabacteroides_distasonis	absent	absent	absent	co-existed	co-existed	co-existed
Megasphaera_unclassified	absent	absent	absent	co-existed	co-existed	co-existed
Ruminococcus_bromii	lean-associated	lean-associated	absent	absent	absent	absent
Acidaminococcus_unclassified	absent	absent	absent	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	absent	absent	absent	co-existed	co-existed	co-existed
Streptococcus_australis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_ovatus	absent	absent	absent	co-existed	co-existed	co-existed
Blautia_hydrogenotrophica	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Roseburia_hominis	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Eubacterium_cylindroides	absent	absent	absent	lean-associated	lean-associated	lean-associated
Clostridium_bolteae	absent	absent	absent	co-existed	co-existed	co-existed
Actinomyces_odontolyticus	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_thetaiotaomicron	absent	lean-associated	absent	lean-associated	lean-associated	lean-associated
Alistipes_shahii	co-existed	co-existed	co-existed	lean-associated	lean-associated	lean-associated
Streptococcus_vestibularis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Holdemania_filiformis	absent	absent	absent	lean-associated	lean-associated	lean-associated
Eggerthella_unclassified	absent	absent	absent	co-existed	co-existed	co-existed
Klebsiella_pneumoniae	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroidales_bacterium_ph8	lean-associated	absent	lean-associated	lean-associated	lean-associated	lean-associated
Prevotella_copri	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_anginosus	absent	lean-associated	absent	lean-associated	lean-associated	absent
Lachnospiraceae_bacterium_1_4_56FAA	absent	absent	absent	co-existed	co-existed	co-existed
Bilophila_wadsworthia	absent	absent	absent	co-existed	co-existed	co-existed
Desulfovibrio_piger	co-existed	co-existed	absent	absent	absent	absent

Detailed Breakdown of Four Groups

Species	FDM040W16	FDM040W24	FDM040W4	FDM041W24	FDM041W4	FDM042W16
Collinsella_aerofaciens	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Dorea_formicigenerans	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Dorea_longicatena	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Streptococcus_salivarius	co-existed	co-existed	co-existed	absent	absent	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Eubacterium_ramulus	lean-associated	lean-associated	lean-associated	lean-associated	absent	lean-associated
Coprococcus_catus	lean-associated	lean-associated	lean-associated	lean-associated	absent	co-existed
Roseburia_inulinivorans	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Eubacterium_rectale	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	lean-associated	lean-associated	lean-associated	absent	co-existed	co-existed
Bifidobacterium_longum	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Alistipes_putredinis	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Bacteroides_caccae	lean-associated	absent	lean-associated	co-existed	co-existed	lean-associated
Bacteroides_massiliensis	lean-associated	lean-associated	lean-associated	co-existed	co-existed	co-existed
Bacteroides_stercoris	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Coprococcus_comes	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Clostridium_leptum	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Bacteroides_uniformis	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Escherichia_unclassified	co-existed	co-existed	co-existed	lean-associated	lean-associated	co-existed
Streptococcus_parasanguinis	absent	absent	lean-associated	absent	absent	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_dorei	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Anaerostipes_hadrus	lean-associated	lean-associated	lean-associated	lean-associated	absent	lean-associated
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Eubacterium_ventriosum	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Barnesiella_intestinihominis	lean-associated	lean-associated	lean-associated	co-existed	co-existed	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	lean-associated	lean-associated	absent	lean-associated	lean-associated
Ruminococcus_gnavus	absent	absent	co-existed	absent	co-existed	lean-associated
Parabacteroides_distasonis	absent	absent	absent	co-existed	co-existed	co-existed
Megasphaera_unclassified	absent	absent	lean-associated	co-existed	co-existed	lean-associated
Ruminococcus_bromii	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Acidaminococcus_unclassified	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Adlercreutzia_equolifaciens	absent	co-existed	co-existed	co-existed	co-existed	absent
Streptococcus_australis	absent	absent	absent	absent	absent	co-existed
Bacteroides_ovatus	absent	absent	absent	co-existed	co-existed	absent
Blautia_hydrogenotrophica	absent	co-existed	co-existed	absent	co-existed	lean-associated
Roseburia_hominis	lean-associated	absent	absent	co-existed	co-existed	absent
Eubacterium_cylindroides	absent	lean-associated	lean-associated	lean-associated	lean-associated	absent
Clostridium_bolteae	lean-associated	absent	lean-associated	absent	co-existed	co-existed
Actinomyces_odontolyticus	absent	absent	absent	absent	absent	co-existed
Bacteroides_thetaiotaomicron	lean-associated	lean-associated	absent	co-existed	co-existed	lean-associated
Alistipes_shahii	lean-associated	lean-associated	absent	co-existed	co-existed	absent
Streptococcus_vestibularis	co-existed	co-existed	co-existed	absent	absent	absent
Holdemania_filiformis	lean-associated	lean-associated	lean-associated	absent	co-existed	co-existed
Eggerthella_unclassified	absent	co-existed	co-existed	absent	co-existed	co-existed
Klebsiella_pneumoniae	absent	absent	absent	absent	absent	absent
Bacteroidales_bacterium_ph8	lean-associated	lean-associated	lean-associated	co-existed	co-existed	absent
Prevotella_copri	absent	absent	absent	absent	absent	co-existed
Streptococcus_anginosus	absent	absent	lean-associated	absent	absent	absent
Lachnospiraceae_bacterium_1_4_56FAA	lean-associated	lean-associated	lean-associated	absent	co-existed	co-existed
Bilophila_wadsworthia	lean-associated	lean-associated	lean-associated	lean-associated	absent	co-existed
Desulfovibrio_piger	absent	absent	absent	absent	absent	absent

Detailed Breakdown of Four Groups

Species	FDM042W24	FDM042W4	FDM043W16	FDM043W24	FDM043W4	FDM046W16
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Lachnospiraceae_bacterium_5_1_63FAA	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	absent	co-existed	co-existed	lean-associated
Eubacterium_rectale	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	absent	absent	lean-associated	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	lean-associated	lean-associated	co-existed	co-existed	co-existed	lean-associated
Bacteroides_caccae	lean-associated	lean-associated	lean-associated	lean-associated	absent	co-existed
Bacteroides_massiliensis	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Bacteroides_stercoris	lean-associated	lean-associated	absent	co-existed	co-existed	lean-associated
Coprococcus_comes	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Clostridium_leptum	lean-associated	lean-associated	absent	co-existed	co-existed	lean-associated
Oscillibacter_unclassified	lean-associated	lean-associated	absent	co-existed	co-existed	lean-associated
Bacteroides_uniformis	absent	lean-associated	absent	co-existed	co-existed	co-existed
Escherichia_unclassified	co-existed	co-existed	absent	lean-associated	lean-associated	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	absent	co-existed	absent	co-existed	co-existed	lean-associated
Bacteroides_dorei	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Anaerostipes_hadrus	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	absent
Bifidobacterium_adolescentis	absent	absent	absent	absent	absent	lean-associated
Eubacterium_ventriosum	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	absent
Barnesiella_intestinihominis	lean-associated	absent	co-existed	co-existed	co-existed	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	lean-associated	absent	absent	absent	absent
Ruminococcus_gnavus	lean-associated	lean-associated	absent	co-existed	co-existed	co-existed
Parabacteroides_distasonis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Megasphaera_unclassified	lean-associated	lean-associated	absent	absent	absent	lean-associated
Ruminococcus_bromii	lean-associated	lean-associated	absent	absent	co-existed	absent
Acidaminococcus_unclassified	co-existed	co-existed	absent	absent	absent	lean-associated
Adlercreutzia_equolifaciens	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Streptococcus_australis	co-existed	co-existed	absent	absent	absent	co-existed
Bacteroides_ovatus	absent	lean-associated	co-existed	co-existed	co-existed	co-existed
Blautia_hydrogenotrophica	lean-associated	lean-associated	absent	lean-associated	absent	co-existed
Roseburia_hominis	absent	absent	co-existed	co-existed	co-existed	absent
Eubacterium_cylindroides	absent	absent	absent	absent	co-existed	lean-associated
Clostridium_bolteae	co-existed	co-existed	absent	co-existed	co-existed	lean-associated
Actinomyces_odontolyticus	co-existed	co-existed	absent	absent	absent	co-existed
Bacteroides_thetaiotaomicron	absent	absent	absent	co-existed	co-existed	co-existed
Alistipes_shahii	lean-associated	absent	co-existed	co-existed	co-existed	lean-associated
Streptococcus_vestibularis	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Holdemania_filiformis	co-existed	co-existed	absent	co-existed	co-existed	lean-associated
Eggerthella_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Klebsiella_pneumoniae	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Bacteroidales_bacterium_ph8	absent	absent	absent	co-existed	co-existed	absent
Prevotella_copri	co-existed	co-existed	lean-associated	lean-associated	absent	absent
Streptococcus_anginosus	lean-associated	absent	lean-associated	lean-associated	absent	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	co-existed	co-existed	absent	absent	co-existed	co-existed
Bilophila_wadsworthia	absent	absent	co-existed	co-existed	co-existed	co-existed
Desulfovibrio_piger	absent	absent	lean-associated	lean-associated	absent	absent

Detailed Breakdown of Four Groups

Species	FDM046W24	FDM046W4	FDM047W16	FDM047W24	FDM047W4	FDM048W16
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	lean-associated	lean-associated	lean-associated	lean-associated	absent	lean-associated
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	absent	absent	lean-associated	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	lean-associated	lean-associated	absent	co-existed
Eubacterium_ramulus	co-existed	co-existed	lean-associated	lean-associated	absent	co-existed
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	co-existed	co-existed	co-existed	lean-associated
Eubacterium_rectale	co-existed	co-existed	lean-associated	lean-associated	absent	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	absent	absent	lean-associated	lean-associated	lean-associated	co-existed
Alistipes_putredinis	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Bacteroides_caccae	co-existed	co-existed	lean-associated	lean-associated	absent	lean-associated
Bacteroides_massiliensis	lean-associated	lean-associated	co-existed	co-existed	co-existed	lean-associated
Bacteroides_stercoris	lean-associated	lean-associated	co-existed	co-existed	absent	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Clostridium_leptum	absent	absent	co-existed	co-existed	co-existed	co-existed
Oscillibacter_unclassified	lean-associated	lean-associated	lean-associated	absent	absent	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Escherichia_unclassified	absent	co-existed	co-existed	co-existed	co-existed	absent
Streptococcus_parasanguinis	co-existed	co-existed	absent	absent	absent	co-existed
Bifidobacterium_pseudocatenulatum	absent	absent	lean-associated	lean-associated	absent	co-existed
Bacteroides_dorei	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Anaerostipes_hadrus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_adolescentis	absent	absent	lean-associated	lean-associated	lean-associated	co-existed
Eubacterium_ventriosum	co-existed	co-existed	lean-associated	lean-associated	absent	lean-associated
Barnesiella_intestinihominis	absent	lean-associated	lean-associated	absent	absent	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	absent	lean-associated	co-existed	co-existed	co-existed	lean-associated
Ruminococcus_gnavus	co-existed	co-existed	lean-associated	lean-associated	absent	co-existed
Parabacteroides_distasonis	co-existed	co-existed	co-existed	co-existed	absent	absent
Megasphaera_unclassified	absent	absent	co-existed	co-existed	co-existed	lean-associated
Ruminococcus_bromii	absent	co-existed	co-existed	co-existed	co-existed	absent
Acidaminococcus_unclassified	absent	absent	lean-associated	lean-associated	absent	lean-associated
Adlercreutzia_equolifaciens	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_australis	co-existed	co-existed	absent	absent	absent	lean-associated
Bacteroides_ovatus	co-existed	co-existed	lean-associated	lean-associated	absent	co-existed
Blautia_hydrogenotrophica	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Roseburia_hominis	lean-associated	absent	co-existed	co-existed	co-existed	co-existed
Eubacterium_cylindroides	absent	absent	co-existed	co-existed	co-existed	lean-associated
Clostridium_bolteae	lean-associated	absent	co-existed	co-existed	absent	absent
Actinomyces_odontolyticus	absent	absent	co-existed	co-existed	co-existed	lean-associated
Bacteroides_thetaiotaomicron	absent	co-existed	lean-associated	lean-associated	absent	co-existed
Alistipes_shahii	lean-associated	lean-associated	lean-associated	lean-associated	absent	co-existed
Streptococcus_vestibularis	lean-associated	absent	absent	absent	absent	co-existed
Holdemania_filiformis	absent	absent	co-existed	co-existed	co-existed	co-existed
Eggerthella_unclassified	co-existed	co-existed	absent	absent	co-existed	absent
Klebsiella_pneumoniae	absent	lean-associated	co-existed	co-existed	co-existed	absent
Bacteroidales_bacterium_ph8	absent	absent	co-existed	co-existed	absent	lean-associated
Prevotella_copri	absent	absent	lean-associated	lean-associated	absent	lean-associated
Streptococcus_anginosus	absent	absent	co-existed	co-existed	co-existed	absent
Lachnospiraceae_bacterium_1_4_56FAA	co-existed	co-existed	absent	absent	absent	absent
Bilophila_wadsworthia	co-existed	co-existed	absent	absent	absent	co-existed
Desulfovibrio_piger	co-existed	co-existed	lean-associated	lean-associated	absent	lean-associated

Detailed Breakdown of Four Groups

Species	FDM048W24	FDM048W4	FDM049W16	FDM049W24	FDM049W4	FDM050W16
Collinsella_aerofaciens	co-existed	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	absent	lean-associated	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Eubacterium_ramulus	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Coprococcus_catus	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Eubacterium_rectale	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	co-existed	co-existed	lean-associated	lean-associated	absent	co-existed
Bacteroides_caccae	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Bacteroides_massiliensis	absent	lean-associated	absent	co-existed	absent	lean-associated
Bacteroides_stercoris	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Coprococcus_comes	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Clostridium_leptum	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	absent	absent	co-existed
Escherichia_unclassified	absent	lean-associated	absent	co-existed	absent	absent
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	co-existed	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Bacteroides_dorei	absent	lean-associated	co-existed	co-existed	co-existed	absent
Anaerostipes_hadrus	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Bifidobacterium_adolescentis	co-existed	co-existed	lean-associated	absent	lean-associated	co-existed
Eubacterium_ventriosum	lean-associated	absent	co-existed	absent	absent	lean-associated
Barnesiella_intestinihominis	absent	lean-associated	lean-associated	absent	absent	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	lean-associated	absent	co-existed	absent	co-existed	co-existed
Ruminococcus_gnavus	co-existed	absent	co-existed	co-existed	co-existed	co-existed
Parabacteroides_distasonis	absent	absent	absent	lean-associated	absent	absent
Megasphaera_unclassified	lean-associated	lean-associated	co-existed	absent	absent	co-existed
Ruminococcus_bromii	absent	lean-associated	co-existed	absent	co-existed	absent
Acidaminococcus_unclassified	lean-associated	absent	lean-associated	absent	absent	lean-associated
Adlercreutzia_equolifaciens	co-existed	co-existed	co-existed	absent	absent	co-existed
Streptococcus_australis	lean-associated	lean-associated	absent	lean-associated	absent	absent
Bacteroides_ovatus	co-existed	co-existed	lean-associated	absent	absent	co-existed
Blautia_hydrogenotrophica	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Roseburia_hominis	absent	co-existed	co-existed	absent	absent	lean-associated
Eubacterium_cylindroides	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Clostridium_bolteae	absent	co-existed	absent	lean-associated	absent	absent
Actinomyces_odontolyticus	absent	absent	co-existed	absent	co-existed	absent
Bacteroides_thetaiotaomicron	co-existed	co-existed	lean-associated	absent	absent	co-existed
Alistipes_shahii	absent	co-existed	lean-associated	absent	absent	co-existed
Streptococcus_vestibularis	absent	co-existed	co-existed	absent	co-existed	co-existed
Holdemania_filiformis	absent	absent	co-existed	absent	co-existed	absent
Eggerthella_unclassified	co-existed	co-existed	co-existed	absent	co-existed	absent
Klebsiella_pneumoniae	absent	co-existed	absent	co-existed	co-existed	absent
Bacteroidales_bacterium_ph8	absent	lean-associated	lean-associated	absent	absent	lean-associated
Prevotella_copri	lean-associated	absent	lean-associated	absent	absent	lean-associated
Streptococcus_anginosus	absent	co-existed	co-existed	absent	co-existed	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	absent	co-existed	absent	absent	lean-associated	absent
Bilophila_wadsworthia	absent	co-existed	co-existed	absent	absent	absent
Desulfovibrio_piger	lean-associated	absent	lean-associated	absent	absent	lean-associated

Detailed Breakdown of Four Groups

Species	FDM050W24	FDM050W4	FDM051W16	FDM051W24	FDM051W4	FDM055W16
Collinsella_aerofaciens	absent	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Ruminococcus_obeum	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Dorea_formicigenerans	absent	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Dorea_longicatena	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	absent	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	absent	co-existed	co-existed	co-existed	co-existed	absent
Streptococcus_salivarius	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bilophila_unclassified	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_vulgatus	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_ramulus	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Coprococcus_catus	absent	co-existed	lean-associated	lean-associated	lean-associated	lean-associated
Roseburia_inulinivorans	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	absent	co-existed	lean-associated	lean-associated	lean-associated	lean-associated
Eubacterium_rectale	absent	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Alistipes_putredinis	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_caccae	absent	lean-associated	co-existed	co-existed	co-existed	co-existed
Bacteroides_massiliensis	absent	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Bacteroides_stercoris	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Coprococcus_comes	absent	co-existed	lean-associated	lean-associated	lean-associated	lean-associated
Clostridium_leptum	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Oscillibacter_unclassified	absent	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Bacteroides_uniformis	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_pseudocatenulatum	absent	co-existed	lean-associated	lean-associated	lean-associated	absent
Bacteroides_dorei	absent	co-existed	lean-associated	lean-associated	lean-associated	lean-associated
Anaerostipes_hadrus	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Bifidobacterium_adolescentis	absent	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Eubacterium_ventriosum	absent	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Barnesiella_intestinihominis	absent	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Lachnospiraceae_bacterium_3_1_46FAA	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Ruminococcus_gnavus	absent	co-existed	absent	absent	co-existed	co-existed
Parabacteroides_distasonis	absent	absent	absent	absent	co-existed	co-existed
Megasphaera_unclassified	absent	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Ruminococcus_bromii	absent	absent	co-existed	co-existed	co-existed	absent
Acidaminococcus_unclassified	absent	absent	lean-associated	lean-associated	absent	co-existed
Adlercreutzia_equolifaciens	absent	co-existed	absent	co-existed	absent	co-existed
Streptococcus_australis	absent	absent	absent	absent	lean-associated	absent
Bacteroides_ovatus	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Blautia_hydrogenotrophica	absent	lean-associated	lean-associated	lean-associated	lean-associated	co-existed
Roseburia_hominis	absent	lean-associated	lean-associated	absent	absent	lean-associated
Eubacterium_cylindroides	absent	absent	lean-associated	lean-associated	absent	co-existed
Clostridium_bolteae	absent	co-existed	absent	absent	co-existed	co-existed
Actinomyces_odontolyticus	absent	absent	absent	lean-associated	absent	absent
Bacteroides_thetaiotaomicron	absent	co-existed	co-existed	co-existed	co-existed	lean-associated
Alistipes_shahii	absent	co-existed	lean-associated	lean-associated	lean-associated	lean-associated
Streptococcus_vestibularis	absent	co-existed	absent	co-existed	co-existed	lean-associated
Holdemania_filiformis	absent	co-existed	absent	absent	co-existed	co-existed
Eggerthella_unclassified	absent	co-existed	absent	absent	co-existed	absent
Klebsiella_pneumoniae	lean-associated	absent	absent	absent	absent	lean-associated
Bacteroidales_bacterium_ph8	absent	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Prevotella_copri	absent	absent	lean-associated	lean-associated	absent	lean-associated
Streptococcus_anginosus	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Lachnospiraceae_bacterium_1_4_56FAA	absent	co-existed	absent	absent	co-existed	co-existed
Bilophila_wadsworthia	absent	co-existed	co-existed	co-existed	co-existed	co-existed
Desulfovibrio_piger	absent	absent	lean-associated	lean-associated	absent	lean-associated

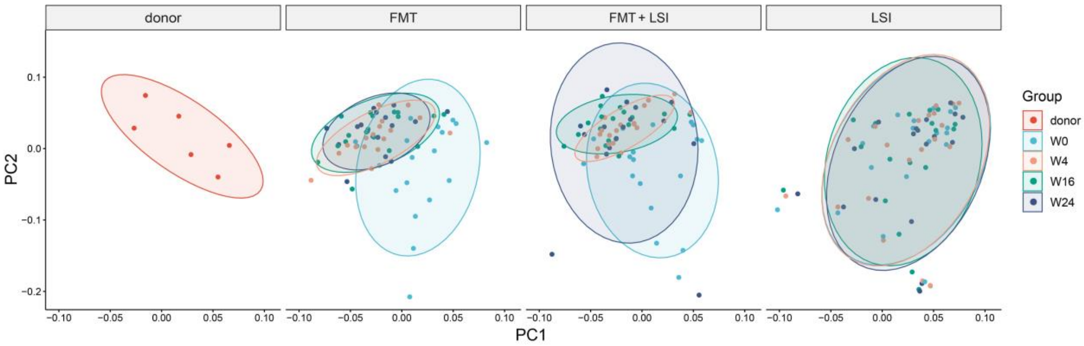
Detailed Breakdown of Four Groups

Species	FDM055W24	FDM055W4	FDM059W16	FDM059W24	FDM059W4	FDM060W16
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	lean-associated	absent	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	co-existed	co-existed	lean-associated	lean-associated	lean-associated	lean-associated
Streptococcus_salivarius	co-existed	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Bilophila_unclassified	co-existed	co-existed	absent	co-existed	co-existed	co-existed
Bacteroides_vulgatus	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Eubacterium_ramulus	lean-associated	lean-associated	co-existed	co-existed	co-existed	lean-associated
Coprococcus_catus	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Roseburia_inulinivorans	co-existed	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Collinsella_tanakaei	lean-associated	lean-associated	co-existed	co-existed	co-existed	lean-associated
Eubacterium_rectale	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	co-existed	co-existed	co-existed	absent	co-existed	lean-associated
Alistipes_putredinis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_caccae	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_massiliensis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_stercoris	lean-associated	lean-associated	co-existed	co-existed	co-existed	co-existed
Coprococcus_comes	lean-associated	absent	co-existed	co-existed	co-existed	lean-associated
Clostridium_leptum	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	absent	co-existed	co-existed
Escherichia_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Bifidobacterium_pseudocatenulatum	absent	lean-associated	co-existed	absent	co-existed	absent
Bacteroides_dorei	lean-associated	absent	absent	lean-associated	lean-associated	co-existed
Anaerostipes_hadrus	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated	lean-associated
Bifidobacterium_adolescentis	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Eubacterium_ventriosum	lean-associated	absent	absent	absent	absent	co-existed
Barnesiella_intestinihominis	co-existed	co-existed	co-existed	co-existed	co-existed	lean-associated
Lachnospiraceae_bacterium_3_1_46FAA	absent	absent	co-existed	co-existed	co-existed	co-existed
Ruminococcus_gnavus	co-existed	co-existed	absent	absent	lean-associated	co-existed
Parabacteroides_distasonis	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Megasphaera_unclassified	co-existed	co-existed	absent	absent	absent	absent
Ruminococcus_bromii	absent	absent	absent	absent	absent	co-existed
Acidaminococcus_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Adlercreutzia_equolifaciens	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_australis	co-existed	absent	absent	absent	absent	co-existed
Bacteroides_ovatus	co-existed	co-existed	lean-associated	lean-associated	lean-associated	co-existed
Blautia_hydrogenotrophica	co-existed	absent	lean-associated	absent	absent	co-existed
Roseburia_hominis	lean-associated	absent	lean-associated	lean-associated	absent	co-existed
Eubacterium_cylindroides	co-existed	co-existed	co-existed	co-existed	co-existed	co-existed
Clostridium_bolteae	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Actinomyces_odontolyticus	co-existed	co-existed	absent	absent	absent	co-existed
Bacteroides_thetaiotaomicron	lean-associated	absent	co-existed	co-existed	co-existed	absent
Alistipes_shahii	lean-associated	absent	co-existed	co-existed	co-existed	co-existed
Streptococcus_vestibularis	absent	absent	lean-associated	lean-associated	lean-associated	absent
Holdemania_filiformis	co-existed	co-existed	co-existed	co-existed	co-existed	absent
Eggerthella_unclassified	absent	co-existed	co-existed	absent	co-existed	absent
Klebsiella_pneumoniae	lean-associated	lean-associated	co-existed	co-existed	co-existed	absent
Bacteroidales_bacterium_ph8	absent	lean-associated	absent	absent	absent	co-existed
Prevotella_copri	lean-associated	absent	lean-associated	lean-associated	absent	lean-associated
Streptococcus_anginosus	co-existed	co-existed	absent	absent	absent	lean-associated
Lachnospiraceae_bacterium_1_4_56FAA	co-existed	co-existed	absent	absent	absent	absent
Bilophila_wadsworthia	co-existed	co-existed	absent	absent	absent	absent
Desulfovibrio_piger	lean-associated	absent	co-existed	co-existed	co-existed	lean-associated

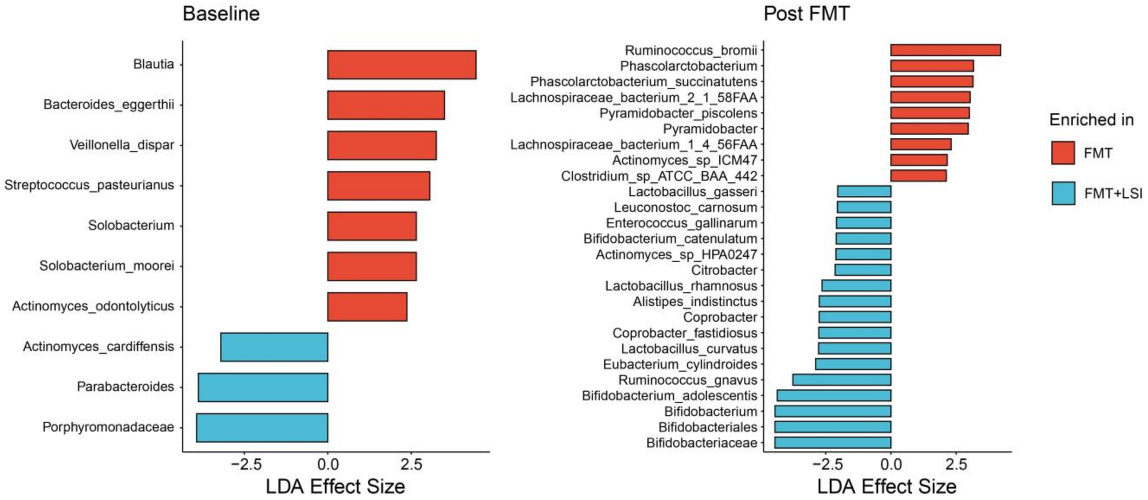
Detailed Breakdown of Four Groups

Species	FDM060W24	FDM060W4	FDM061W16	FDM061W24	FDM061W4
Collinsella_aerofaciens	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_obeum	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_torques	co-existed	co-existed	co-existed	co-existed	co-existed
Ruminococcus_sp_5_1_39BFAA	co-existed	co-existed	co-existed	co-existed	co-existed
Subdoligranulum_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_hallii	co-existed	co-existed	co-existed	co-existed	co-existed
Faecalibacterium_prausnitzii	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_formicigenerans	co-existed	co-existed	co-existed	co-existed	co-existed
Dorea_longicatena	co-existed	co-existed	co-existed	co-existed	co-existed
Escherichia_coli	co-existed	co-existed	co-existed	co-existed	co-existed
Parabacteroides_merdae	co-existed	co-existed	co-existed	absent	co-existed
Lachnospiraceae_bacterium_5_1_63FAA	lean-associated	lean-associated	co-existed	co-existed	co-existed
Streptococcus_salivarius	absent	absent	co-existed	co-existed	co-existed
Bilophila_unclassified	co-existed	co-existed	absent	lean-associated	absent
Bacteroides_vulgatus	lean-associated	absent	co-existed	co-existed	co-existed
Eubacterium_ramulus	lean-associated	lean-associated	lean-associated	absent	lean-associated
Coprococcus_catus	co-existed	co-existed	co-existed	absent	co-existed
Roseburia_inulinivorans	co-existed	co-existed	co-existed	co-existed	co-existed
Collinsella_tanakaei	lean-associated	absent	absent	absent	absent
Eubacterium_rectale	co-existed	co-existed	co-existed	co-existed	co-existed
Lachnospiraceae_bacterium_1_1_57FAA	co-existed	co-existed	co-existed	co-existed	co-existed
Bifidobacterium_longum	lean-associated	absent	co-existed	co-existed	co-existed
Alistipes_putredinis	co-existed	co-existed	co-existed	absent	co-existed
Bacteroides_caccae	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_massiliensis	co-existed	co-existed	absent	absent	co-existed
Bacteroides_stercoris	co-existed	co-existed	absent	absent	co-existed
Coprococcus_comes	lean-associated	absent	co-existed	absent	co-existed
Clostridium_leptum	co-existed	co-existed	co-existed	co-existed	co-existed
Oscillibacter_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed
Bacteroides_uniformis	co-existed	co-existed	co-existed	absent	co-existed
Escherichia_unclassified	co-existed	co-existed	co-existed	co-existed	co-existed
Streptococcus_parasanguinis	absent	absent	lean-associated	lean-associated	lean-associated
Bifidobacterium_pseudocatenulatum	absent	co-existed	absent	co-existed	co-existed
Bacteroides_dorei	co-existed	co-existed	absent	absent	absent
Anaerostipes_hadrus	lean-associated	lean-associated	co-existed	absent	co-existed
Bifidobacterium_adolescentis	lean-associated	lean-associated	absent	lean-associated	lean-associated
Eubacterium_ventriosum	co-existed	co-existed	co-existed	co-existed	co-existed
Barnesiella_intestinihominis	lean-associated	lean-associated	co-existed	absent	co-existed
Lachnospiraceae_bacterium_3_1_46FAA	co-existed	co-existed	lean-associated	lean-associated	absent
Ruminococcus_gnavus	co-existed	co-existed	absent	co-existed	absent
Parabacteroides_distasonis	co-existed	co-existed	co-existed	co-existed	co-existed
Megasphaera_unclassified	absent	absent	absent	absent	absent
Ruminococcus_bromii	co-existed	co-existed	lean-associated	lean-associated	absent
Acidaminococcus_unclassified	absent	lean-associated	absent	absent	absent
Adlercreutzia_equolifaciens	co-existed	co-existed	absent	lean-associated	absent
Streptococcus_australis	co-existed	co-existed	absent	absent	absent
Bacteroides_ovatus	co-existed	co-existed	absent	absent	absent
Blautia_hydrogenotrophica	co-existed	co-existed	absent	absent	absent
Roseburia_hominis	co-existed	co-existed	co-existed	co-existed	co-existed
Eubacterium_cylindroides	co-existed	co-existed	absent	absent	absent
Clostridium_bolteae	absent	co-existed	absent	co-existed	absent
Actinomyces_odontolyticus	absent	absent	lean-associated	lean-associated	absent
Bacteroides_thetaiotaomicron	co-existed	co-existed	absent	co-existed	absent
Alistipes_shahii	co-existed	co-existed	absent	co-existed	co-existed
Streptococcus_vestibularis	absent	absent	absent	lean-associated	lean-associated
Holdemania_filiformis	absent	co-existed	absent	absent	co-existed
Eggerthella_unclassified	absent	co-existed	absent	co-existed	absent
Klebsiella_pneumoniae	absent	absent	absent	lean-associated	absent
Bacteroidales_bacterium_ph8	co-existed	co-existed	co-existed	absent	co-existed
Prevotella_copri	lean-associated	absent	lean-associated	absent	lean-associated
Streptococcus_anginosus	absent	absent	absent	lean-associated	lean-associated
Lachnospiraceae_bacterium_1_4_56FAA	absent	absent	absent	absent	absent
Bilophila_wadsworthia	absent	absent	absent	lean-associated	absent
Desulfovibrio_piger	lean-associated	absent	co-existed	absent	co-existed

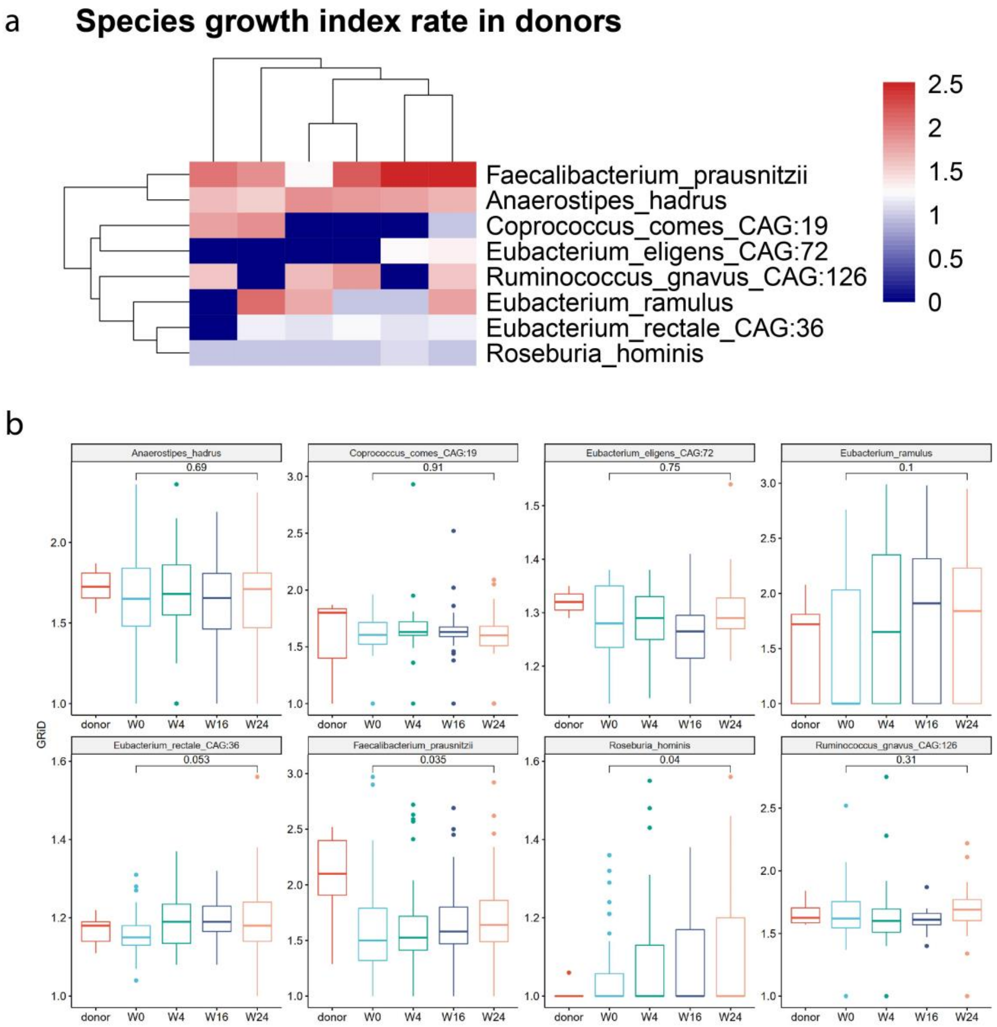
Supplementary figure 3: PCoA depicting changes in butyrate producing microbiotafollowing intervention in 3 groups. PCoA (principal coordinate analysis) calculated based on all PCoA depicting changes in butyrate producing microbiota species. The bacterial composition shifted significantly after intervention in both FMT groups and FMT+LSI group, while sham plus LSI group showed no changes.



Supplementary figure 4: LDA effect size of bacterial species that were differentiallyabundant between FMT alone group and FMT plus LSI group at baseline and post FMT by LEfSe (LDA>2, adjusted p<0.05).

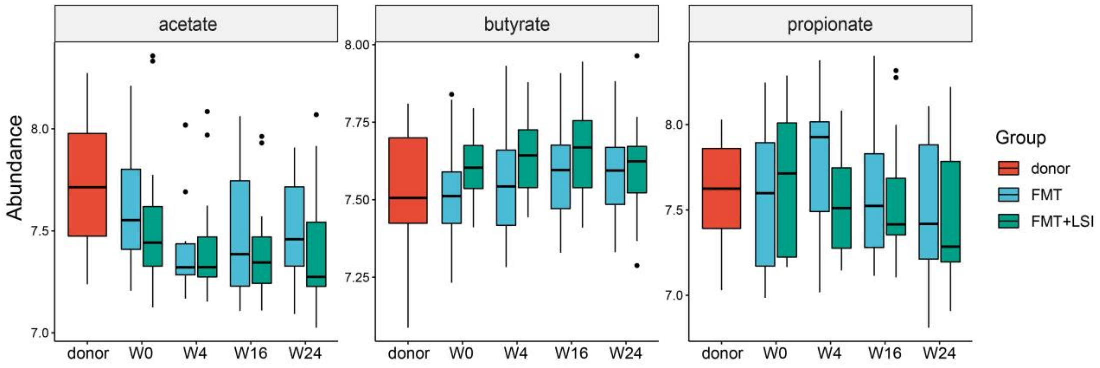


Supplementary figure 5: Growth Rate Index (GRiD) inferred from metagenomicsequencing data. (a) Heatmap depicting the GRiD values The GRiD values of most altered species in lean donors. (b) The GRiD values of most altered species in FMT recipients.

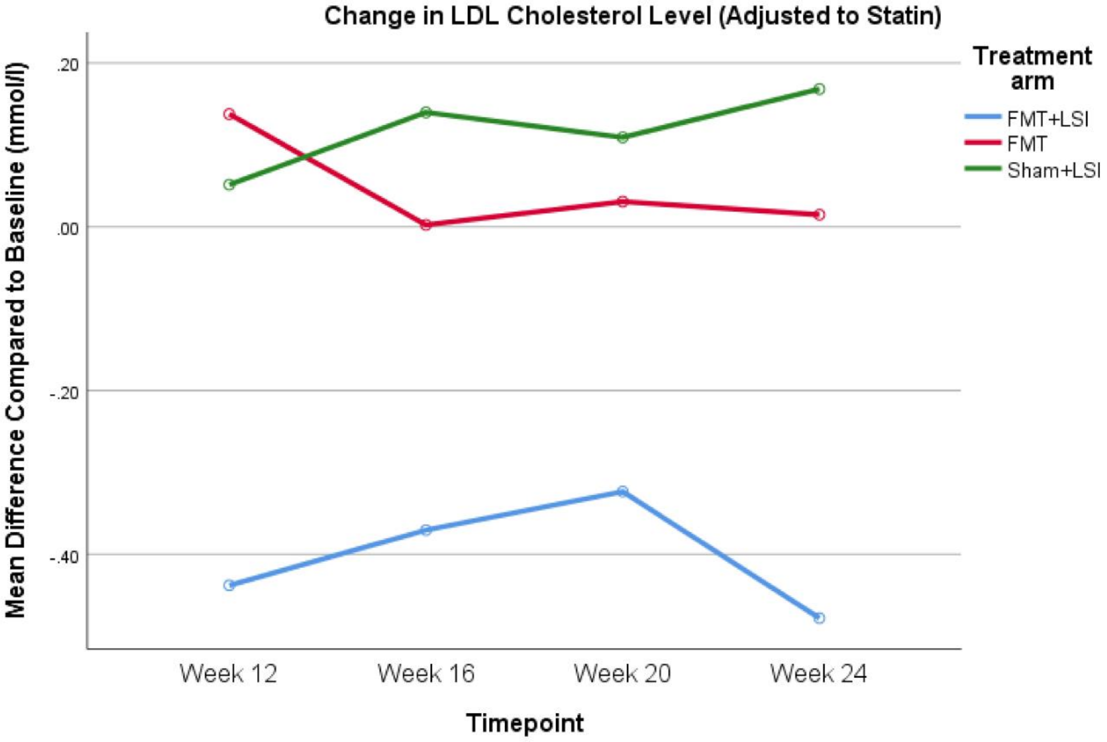


Species growth index rate in donors

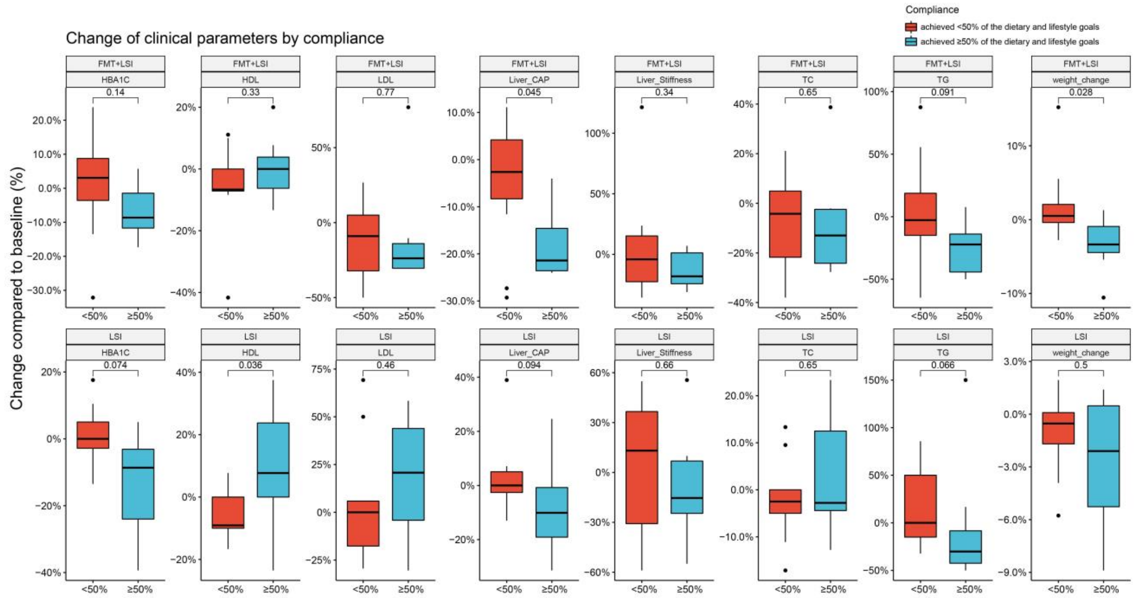
Supplementary figure 6: Boxplot depicting the abundance of short chain fatty acids in lean donors and FMT recipients. Aggregated abundances of acetic, butyric and propionic acids after logarithmic transformation were shown.



Supplementary figure 7: Changes in LDL Cholesterol level post intervention in 3 treatment groups



Supplementary figure 8: Subgroup analysis of clinical parameters between subjects with different compliance to lifestyle modification in FMT plus LSI arm and sham plus LSI arm. Significance between subjects with different compliance to lifestyle modification were calculated by Wilcoxon rank sum test.



Protocol

Title:	A randomised placebo-controlled study of fecal microbiota transplant (FMT) to impact body weight and glycemic control in obese subjects with type 2 diabetes mellitus
Short Title:	FMT-DM-RCT study
Version Number:	FMT-DM-RCT_Protocol_20191015
Principle Investigator:	Prof Siew Chien Ng

Co-Investigator:

Prof. Vincent Wong
Prof. Ronald Ma
Prof. Kwok Wai Enders Ng
Prof. Alice Pik Shan Kong
Dr. Kin Hung Simon Wong
Dr. Elaine Chow
Prof. Sunny Hei Wong
Dr. Rashid Lui
Dr. Moe Kyaw
Dr. Ting Ting Chan
Dr. Ho Shing Louis Lau
Dr. Andrea On Yan Luk
Dr. Yun-ning Elaine Cheung
Dr. Heyson Chi Hey Chan
Dr. Joyce WY Mak
Dr Ting Long Marc Wong
Prof Francis Chan

1. Background

There is a worldwide epidemic of obesity and type 2 diabetes mellitus. The prevalence of obesity and type 2 diabetes mellitus continues to rise at an alarming rate. In China, more than 100 million people are estimated to suffer from diabetes [1]. Similarly, the prevalence of overweight and obese individuals in China has increased by four-fold from 3.7% to 19.0% over two decades [1]. Weight loss is associated with reductions in risk of morbidity and mortality from obesity [2, 3]. Conventional non-pharmacological interventions based on diet and exercise showed limited long-term success in producing sustained weight loss [4]. Although obese subjects with type 2 diabetes mellitus may be treated by medications or by bariatric surgery, these alternatives are limited by incomplete resolution of the diseases, high cost or potential surgical-related morbidity. Further research focusing on increasing effectiveness of interventions and new ways to achieve weight loss in these individuals are needed.

Recently, accumulating evidence supports a role of the enteric microbiota in the pathogenesis of obesity-related insulin resistance. Obesity is associated with changes in the composition of the intestinal microbiota, and the obese microbiome appears to be more efficient in harvesting energy from the diet [5]. Colonization of germ-free mice with an 'obese microbiota' results in a significantly greater increase in total body fat than colonization with a 'lean microbiota', suggesting gut microbiota as an additional contributing factor to the pathophysiology of obesity [6]. Obese and lean phenotypes can also be induced in germ-free mice by transfer of fecal microbiota from human donors [7, 8]. These data have led to the use of microbiota therapeutics as a potential treatment for metabolic syndrome and obesity.

Fecal microbiota transplantation (FMT) represents a clinically feasible way to restore the gut microbial ecology, and has proven to be a breakthrough for the treatment of recurrent *Clostridium difficile* infection [9]. Furthermore, clinical trials are being conducted to evaluate its use for other conditions including inflammatory bowel disease [10], irritable bowel syndrome, diabetes mellitus, non-alcoholic steatohepatitis and hepatic encephalopathy. Early results in human have shown that FMT from lean donor when transplanted into subjects with metabolic syndrome resulted in a significant improvement in insulin sensitivity and an increased in intestinal microbial diversity, including a distinct increase in butyrate-producing bacterial strains [11]. The colonization of donor microbiota lasted for at least 3 months following FMT [12]. The therapy is generally well tolerated and appeared safe [13, 14]. No clinical studies have assessed the efficacy of FMT in obese subjects with type 2 diabetes mellitus. No clinical studies have assessed the microbial transfer and its durability after FMT in obese subjects with type 2 diabetes mellitus. We also assessed the safety and efficacy of FMT in this RCT.

2. Hypothesis

We hypothesize that FMT is superior to lifestyle intervention alone in inducing a sustainable change of lean-associated microbiota (including bacteria and virus) in obese subjects with type 2 diabetes mellitus.

3. Aims

i. To determine changes in microbiota in obese subjects with type 2 diabetes mellitus
ii. To determine microbial composition, function and metabolite changes before and after FMT
iii. To assess the safety of FMT in obese subjects with type 2 diabetes mellitus

4. Study Outcomes

4.1. Primary Outcome

Proportion of subjects with at least 20% lean-associated microbiota in recipients after FMT compared with subjects receiving lifestyle intervention alone up to week 24. Definition:

- A 20% cut-off is used since a previous study observed that FMT led to engraftment of an average of 10% lean-associated microbiota in subjects with metabolic syndrome after one single dose of FMT[12], whereas multiple infusions may enhance the engraftment of lean-associated microbiota.
- A species is considered lean-associated if it is present in donor fecal samples but not present in recipient before FMT.
- A species is considered present if its relative abundance is greater than or equal to 0.01%.

4.2. Secondary Outcome

i. Changes in microbial composition (including bacteriome and virome), function and metabolite at weeks 4, 16, 20 and 24 compated with baseline
ii. Changes in microbiome of stool (including bacteriome and virome) at weeks 4, 16, and 24 compared with baseline
iii. Difference in microbiome (including bacteriome and virome) compared between subjects in different treatment arm
iv. Proportion of microbiome (including bacteriome and virome) derived from recipient, donor or both in subjects who received FMT
v. Difference in microbiome (including bacteriome and virome) compared between subjects who have weight loss and those do not have weight loss
vi. Microbial factors (including bacteriome and virome) that are associated with percentage of body weight loss
vii. Trans-kingdom correlation of microbial engraftment after FMT between bacteriome and virome
viii. Proportion of subjects with serious adverse events compared between treatment arm, especially those related to FMT
ix. Explore changes in fungome microbiota.
x. Proportion of subjects achieving at least 10% reduction in weight at week 52 compared with baseline
xi. Proportion of subjects achieving at least 10% reduction in weight at 24 weeks compared with baseline
xii. Changes in body mass index (BMI) at weeks 24 and 52 compared with baseline
xiii. Changes in liver biochemistry, fasting glucose, fasting lipids, fasting insulin, HBA1c at weeks 24 and 52 compared with baseline
xiv. A 30% decrease in insulin resistance at weeks 24 compared with baseline
xv. Changes in liver stiffness to assess improvement of other metabolic disease weeks 24 compared with baseline

Safety assessments will include the monitoring of adverse events and clinical laboratory testing.

5. Methods

5.1. Study Design

This is a double blinded, placebo-controlled randomized trial. Subjects will be randomized in a blinded fashion in a 1:1:1 ratio. FMT will be done during the first 12 weeks period. Subjects will then be followed up under routine care. There will be a final follow up visit at week 52. Treatment groups are as follow:

- Treatment Group 1 (n = 20): FMT + Lifestyle intervention
- Treatment Group 2 (n = 20): FMT
- Treatment Group 3 (n = 20): Sham + Lifestyle intervention

5.2. Study Population

5.2.1. Obese Subjects with Type 2 Diabetes Mellitus

Subjects who fulfil eligibility criteria in Prince of Wales Hospital will be recruited. Subject records and referral letters will be screened for potential subjects. Potential subjects will be contacted by phone. Subjects will also be invited through advertisements or public announcement. Only subjects meeting inclusion criteria and exclusion criteria will be invited to the study.

Inclusion criteria

1 Age 18-70; and
2 BMI ≥28 kg/m^2 and < 45 kg/m^2; and
3 A diagnosis of Type 2 diabetes mellitus for ≥3 months; and
4 Written informed consent obtained

Exclusion criteria

1 Current pregnancy
2 Use of any weight loss medications in the preceding 1 year
3 Known history or concomitant significant gastrointestinal disorders (including Inflammatory Bowel Disease, current colorectal cancer, current GI infection)
4 Known history or concomitant significant food allergies
5 Immunosuppressed subjects
6 Known history of severe organ failure (including decompensated cirrhosis), inflammatory bowel disease, kidney failure, epilepsy, acquired immunodeficiency syndrome
7 Current active sepsis
8 Active malignant disease in recent 2 years
9 Known contraindications to oesophago-gastro-duodenoscopy (OGD)
10 Use of probiotic or antibiotics in recent 3 months
11 On Sodium-glucose co-transporter-2 inhibitors or Glucagon-like peptide-1 receptor agonists at randomization
12 On Proton-pump inhibitor at randomization

Prohibited medications

No antibiotics, probiotic or prebiotic preparations, Sodium-glucose co-transporter-2 (SGLT2) inhibitors, Glucagon-like peptide-1 (GLP-1) receptor agonists or Proton-pump inhibitor (PPI) will be permitted during the study.

Subjects with intake of prohibited medication during study period will remain in study and outcome will be assessed. The reported intake of prohibited medication will be recorded and documented.

5.2.2. Stool Donors

Stool donated from donors (BMI < 23kg/m^2) of two independent studies [(CRE. 2016.033) Study title: Development of donor screening and systematic stool bank for Fecal Microbiota Transplantation (Stool bank study)] or [(CRE. 2016.707) Study title: Stool Biobank of the Faculty of Medicine, The Chinese University of Hong Kong] will be processed and used for FMT infusion. In the aforementioned studies, volunteers from general population including spouses or partners, first-degree relatives, other relatives, friends and others who are known or unknown to the potential subjects that met eligibility criteria will be invited for screening laboratory test. A series of laboratory tests for infectious diseases and interviews will be done. Stool from the eligible donors will be used in this study. Subjects may receive stools from single or multiple donors whose identity may not be made available to the subjects.

5.3. Study Procedures

5.3.1. Fecal Microbiota Transplant (FMT) / Sham

FMT: Frozen or fresh stool from donors of Stool bank studies will be used in this protocol depending on donor stool availability. FMT solution will be freshly prepared on the same day of infusion using stool from single donor or mixing of stool from multiple donors. Feces will be diluted with sterile saline (0.9%). This solution will be blended and strained with filter. The resulting supernatant will then be used directly as fresh FMT solution or stored as frozen FMT solution for future FMT.

Sham: Sterile saline (0.9%) will be used as sham.

Procedures for Infusion: 100-200ml of FMT solution or sterile saline will be infused over 2-3 minutes into the distal duodenum or jejunum via OGD. After infusion, subjects will be monitored for 1 hour before discharged.

5.3.2. Lifestyle intervention / Dietary advices and Dietary Assessment

Lifestyle intervention: Subjects in FMT + lifestyle intervention arm or Sham + lifestyle interventionarm will be seen by a dietitian at week 0, 1, 2, 4, 6, 8 and 12. Dietitian will guide and correct subjects in terms of their dietary habits, physical activity patterns, and other lifestyle habits. Subjects will record their dietary history for 7 days for consultation of dietitian.

Dietary advices: All subjects will receive dietary advices from physician or dietitian during follow up visits.

Dietary assessment: Dietary history will be collected for all subjects at week 0, 4, 8, 12, 24 and 52 to collect data on dietary habits. Subjects will complete a 3 day dietary record and may be interviewed for further dietary information.

5.3.3. Demographics and Medical History

Demographics and medical history such as sex, age, smoking and alcohol status, disease onset, co-morbid illness, drug history, clinical test results will be obtained by reviewing of subject medical notes and interview with subjects by doctors and research staff.

5.3.4. Obesity Measurement

Body weight, height, BMI, waist and hip circumference, waist to hip ratio, blood pressure and heart rate will be measured.

5.3.5. Blood Sugar Level

If subjects have a routine practice of measuring blood sugar at home (e.g. by hemastix), the self-reported readings will be recorded.

5.3.6. Clinical Laboratory Test

Blood samples will be collected for complete blood count (CBC), renal function test (RFT), liver function test (LFT), C-reactive protein (CRP) and magnesium. Fasting serum concentrations of glucose, insulin (only at baseline and week 24), total cholesterol, low-density-lipoprotein (LDL) cholesterol, high-density-lipoprotein (HDL) cholesterol, triglycerides and haemoglobin A1c (HbA1C) will be measured. For baseline, results of these tests will be used directly if tests were done within 7 days before screening visit.

5.3.7. Transient Elastrography (TE)

Liver stiffness and liver fat will be measured at week 0 and week 24, using transient elastrography. Transient elastrography is a non-invasive test of liver fibrosis and liver fat that is quick and easy to perform and has a high degree of subject acceptance. The liver stiffness measurement (LSM) score will be represented by the median of 10 measurements and will be considered reliable only if at least 10 successful acquisitions are obtained. Liver fat will be estimated using controlled attenuation parameter (CAP) measurement. The CAP score will be represented by the median value. Subject can choose to opt-out of this measurement and, unless subject has indicated, will not be withdrawn from the study.

5.3.8. Hyperinsulinemic Euglycemic Clamp

Insulin sensitivity will be measured at week 0 and week 24 using hyperinsulinemic euglycemic clamp. Prior to each clamp, pregnancy test will be performed for all female subjects of child-bearing potential, except postmenopausal subjects and sterilized subjects. Pregnant subjects will be withdrawn from this study (Refer to 5.6).

Electrocardiography (ECG) will be done for all subjects prior to clamp at week 0. Subjects with clinically significant abnormality on ECG at screening as judged by investigator will not go on to clamp. However, these subjects can still remain in the study.

Subjects will be fasted 10-12 hours overnight. Diabetic medications will be withheld for 1-2 days before. The hyperinsulinemic euglycemic clamps will be conducted at the Phase 1 Clinical Trial Centre, Prince of Wales Hospital. A low dose intravenous insulin infusion will be given to achieve baseline blood glucose of around 5mmol/L. An intravenous cannula will be placed in the antecubital fossa or large upper limb vein for infusion of insulin and 20% dextrose. A second intravenous cannula will be inserted into the dorsal or wrist vein in a retrograde fashion for glucose sampling and placed in an insulated warm box heated to 55℃ for arterialization of venous blood. At the start of the hyperinsulinaemic euglycemic clamp (Time 0), a primed infusion of intravenous insulin will be given (60mU/m2/min). 20% dextrose is also given intravenously at a variable rate, titrated according to arterialized blood glucose (1-2ml of blood) measured every 5 minutes. Blood glucose concentrations will be maintained at a target of 5mmol for 2 hours. Vital signs and ECG will be monitored during the clamp study. The M value, measure of peripheral insulin sensitivity, will be determined from average glucose infusion rates when blood glucose is at a steady-state. Plasma insulin will be collected at Time 100, Time 110 and Time 120 minutes[18]. At the end of the clamp, insulin will be switched off and 20% dextrose continued for 30 minutes. Blood glucose will be monitored for at least 30 minutes to ensure it remains within stable levels.

Subject can choose to opt-out of this measurement and, unless subject has indicated, will not be withdrawn from the study.

5.4. Randomization, Blind Maintenance and Breaking Treatment Codes

5.4.1. Randomization

The randomization code will be computer-generated, and randomization will be performed in blocks of twelve. Subjects will be randomized in sequence according to the randomization code.

5.4.2. Blind Maintenance

To maintain study procedure blinding, endoscopists and staffs involved in the FMT or sham preparation and infusion will be unblinded. Subjects will be randomized and arranged to have infusion according to randomization table which is only accessible by the unblinded personnel (Figure 1). Procedures including screening and follow up assessment at treatment visits can be performed by blinded or unblinded personnel. Physicians and staffs that are blinded to the study procedure assignment of the subject will assess subject at post treatment visits and final follow up visit.

5.4.3. Breaking treatment codes

In the event of a medical emergency where breaking the blind is required to provide medical care to the subject, the investigators may obtain treatment assignment from unblinded study team. Treatment assignment should remain blinded unless that knowledge is necessary to determine subject emergency medical care. The rationale for unblinding should be clearly explained and documented, along with the date of treatment unblinding. All subjects will be followed until week 52 unless consent to do so is specifically withdrawn by the subject.

5.5. Study Visits (refer to Figure 2: Schematic trial design)

5.5.1. Screening (Day-21 to Day 0)

Demographics and medical history, obesity measurement will be obtained from subject medical notes and referral letters. Subjects fulfilling inclusion and exclusion criteria will be invited and informed consent will be obtained. Obesity measurement, clinical laboratory test, transient elastrography and ECG (as part of hyperinsulinemic euglycemic clamp) will be done either at screening or at week 0 prior to FMT/sham infusion.

5.5.2. Treatment Visits (Week 0 to Week 12)

Subjects who meet all eligibility criteria at screening will be enter to study. If obesity measurement, clinical laboratory test, transient elastrography or ECG (as part of hyperinsulinemic euglycemic clamp) has not yet been done at screening, these can be completed at week 0 prior to FMT or sham infusion. Hyperinsulinemic euglycemic clamp will also be done at week 0 prior to FMT or sham infusion.

Subjects will be randomized to either Treatment Group 1, 2 or 3 in a 1:1:1 ratio. All subjects will then receive either duodenal infusion of FMT or normal saline. Obesity measurement will be repeated at week 4, week 8 and week 12. Clinical laboratory test (except fasting serum concentration of insulin) will be repeated at week 12.

Subjects will also be assessed for any adverse events / serious adverse events for safety monitoring at each treatment visits.

Visits for subjects receiving lifestyle intervention will be different. For subjects randomized to Treatment Group 1 and Treatment Group 3, they will receive lifestyle intervention by seeing dietitian at week 0, 1, 2, 4, 6, 8 and 12.

5.5.3. Post Treatment Visits (Week 16 to Week 24)

Following completion of total 4 infusions, subjects will be assessed at week 16, week 20 and week 24. During each of these visits, subjects will receive obesity measurement and will also be assessed for any adverse events / serious adverse events for safety monitoring. Clinical laboratory test, transient elastrography and/or hyperinsulinemic euglycemic clamp will be done at week 24. After completing follow up at week 24, subjects will follow routine care.

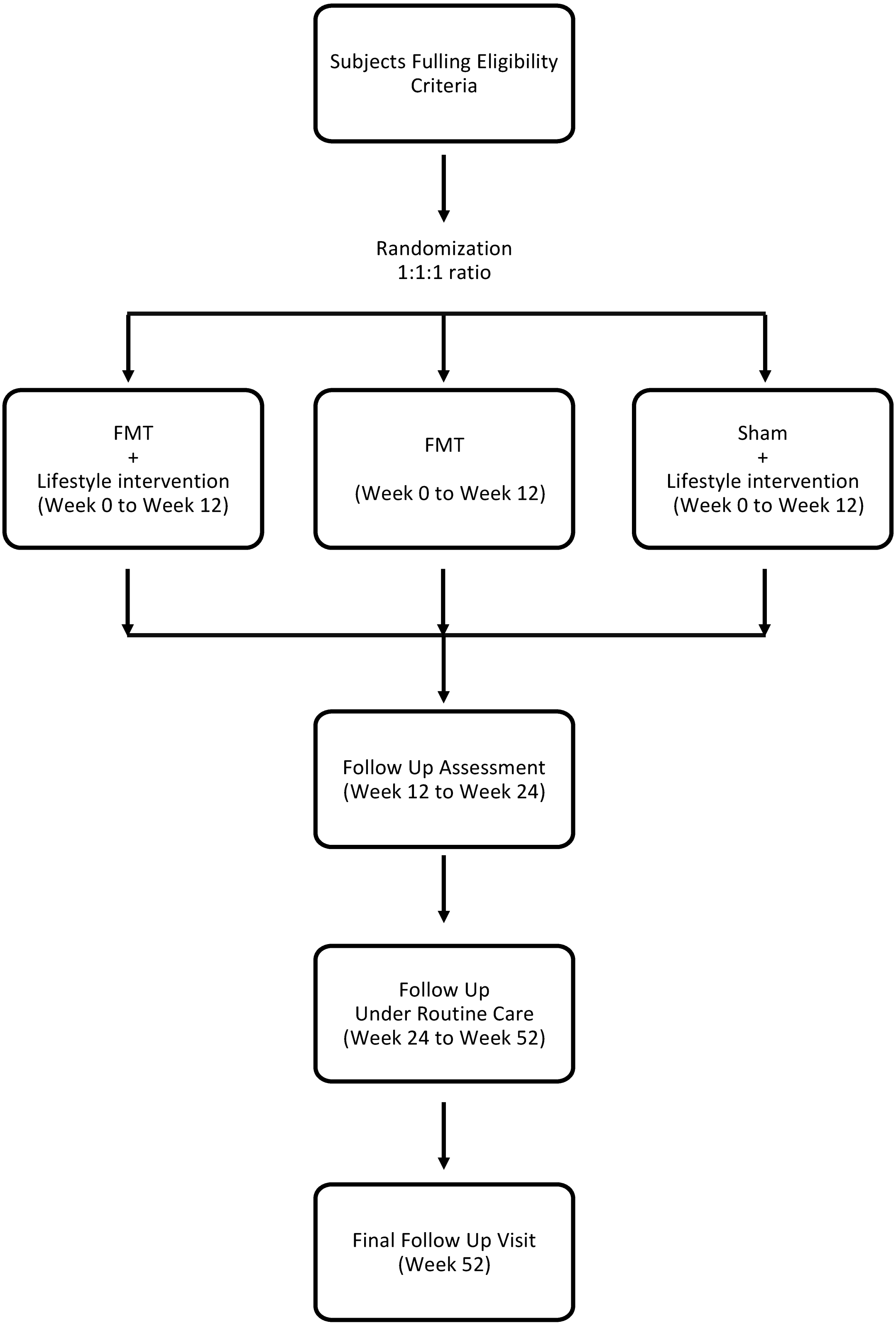
5.5.4. Final Follow Up Visit (Week 52) or Early Termination Visit

At week 52, subject will come back for a final follow up visit to assess long term outcome. If subject's study participation is discontinued at any time before final follow up visit, an early termination visit will be arranged if possible.

During this visit, obesity measurement and clinical laboratory test (except fasting serum concentration of insulin) will be performed.

If subjects cannot come back in person for final follow up visit or early termination visit, a phone interview will be arranged instead. During this follow up, we will interview subjects regarding information including treatment regimen in between week 24 and 52, and changes in weight over time. Subjects will be asked to self-measure waist and hip circumference and such data will be collected. Subject hospital record will also be reviewed and data may be extracted directly from hospital record.

Figure 1: Study Procedure



5.6. Pregnancy

Pregnancy test will be performed for all female subjects of child-bearing potential, except postmenopausal subjects and sterilized subjects. Female of childbearing potential should take contraception measures throughout the study period. Female subjects will be advised to inform study team immediately in case of pregnancy. If female subjects become pregnant during study period, they will be withdrawn from the study. An early termination visit will be arranged upon acknowledgement of subjects' pregnancy if possible.

5.7. Pretreatment Events (PTE), Adverse Events (AE) and Serious Adverse Events (SAE)

5.7.1. Medically Anticipated Clinical Events for Post-FMT

It is known that FMT is associated with certain characteristic signs and symptoms (listed below)[7], which are mild and usually will resolve within 3 days after FMT. These signs and symptoms will not be collected as AEs, unless they persist for more than 3 days or worsen.

- Sore throat
- Fever
- Increase of CRP
- Diarrhea
- Increased in stool frequency
- Loose stool
- Abdominal discomfort
- Abdominal distention
- Abdominal pain
- Abdominal fullness
- Bloating
- Belching
- Cramping
- Gassiness
- Fatigue
- Vomiting
- Excessive flatulence
- Constipation
- Decreased appetite
- Headache
- Nausea
- Borborygmus

5.7.2. Medically Anticipated Clinical Events for Type 2 Diabetes Mellitus

There are fluctuation of signs and symptoms for Diabetes Mellitus patients (listed below), mild signs and symptoms will not be collected as AEs unless they worsen.

- Hypoglycemia
- Hyperglycemia
- Sweating
- Trembling
- Difficulty thinking
- Dizziness
- Weakness
- Malaise
- Poor appetite

5.7.3. Severity

The severity grading of AEs will be assessed as Grade 1, 2, 3, 4 or 5 using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 Grading Scale, which can be found at:
http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.

5.7.4. Collection and Reporting of PTE, AE and SAE

Collection of PTEs will commence once the subject signed informed consent form and continue until the subject receive first infusion of FMT or screen failure. For subjects who discontinue prior to first infusion of FMT, PTEs are collected until the subject discontinues study participation. PTEs fulfilling definition of SAEs according to ICH GCP will be considered and reported as SAEs.

Collection of AEs and SAEs will commence from the time that the subject receive first infusion of FMT. Routine collection of AEs will continue until 24 weeks (study completion). Since subjects will be followed up under routine care after 24 weeks, AEs occur between 24 weeks and 52 weeks will not be collected.

6. Blood, Urine, Biopsies and Stool Sample for Fecal Microbiota and Metabolite Analysis

Metabolism has an essential role in biological systems; and metabolites represent the end products of this important process from a cell of a certain physiological status. Metabolic profiling by identification and quantification of these metabolites may pinpoint components important to investigate the characteristics of subjects.

Study blood (not more than 20ml), and urine sample will be collected at week 0, 4, 12, 24 and 52. Study stool samples will also be collected for research purpose at week 0, 4, 8, 12, 16, 20, 24 and 52. Study biopsies from the Antrum and the Body (2 biopsies from each location) will be collected during the OGD procedure at week 0, 4, 8, and 12. These samples will be stored for future studies, including genetics and microbiota analysis.

All study blood, urine, stool and biopsies are optional. Subjects who do not wish to have study specimen collected can still remain in the study.

Figure 2: Schematic trial design

Study visit (day/week)	**Day**	**Week**										
	-21 to 0	**0**	**1**	**2**	**4**	**6**	**8**	**12**	**16**	**20**	**24**	**52**
Window period (day)		±5	±3	±3	±5	±3	±5	±5	±5	±5	±5	±10
	Screening	**Treatment visits**							**Post treatment visits**			**Final follow up visit**
Informed consent	**x**											
Inclusion and exclusion criteria	**x**											
Demographics and medical history	**x**											
Pregnancy test (if applicable)	**x**											
Pre-treatment events	**x**											
Adverse events/serious adverse events		**x**			**x**		**x**	**x**	**x**	**x**	**x**	**x**
Infusion of FMT/sham		**x**			**x**		**x**	**x**				
Lifestyle intervention [2]		**x**	**x**	**x**	**x**	**x**	**x**	**x**				
Dietary advices		**x**			**x**		**x**	**x**	**x**	**x**	**x**	**x**
Dietary assessment		**x**			**x**		**x**	**x**			**x**	**x**
Obesity measurement (Body weight and height, waist circumference, waist to hip ratio, blood pressure, heart rate)	**X**^{3}	**x**^{3}			**x**		**x**	**x**	**x**	**x**	**x**	**x**
Clinical laboratory test												
1. CBC, LFT, RFT, CRP, magnesium	**X**^{3}	**X**^{3}						**x**	**x**	**x**	**x**	**x**
2. Fasting serum concentration (Glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides)	**X**^{3}	**X**^{3}						**x**	**x**	**x**	**x**	**x**

Study visit (day/week)	**Day**	**Week**										
	-21 to 0	**0**	**1**	**2**	**4**	**6**	**8**	**12**	**16**	**20**	**24**	**52**
Window period (day)		±5	±3	±3	±5	±3	±5	±5	±5	±5	±5	±10
	Screening	**Treatment visits**							**Post treatment visits**			**Final follow up visit**
3. HbA1C	X^3	X^3							x		x	x
4. Fasting serum concentration (insulin)	x^3	x^3									x	
Study blood and urine	X^3	x^3			x			x			x	x
Study stool for microbiota	x^3	x^3			x		x	x	x	x	x	x
Study biopsies (2x Antrum, 2x Body)		x			x		x	x				
Hyperinsulinemic Euglycemic Clamp												
1. ECG (if applicable)	$X^{1,3}$	$x^{1,3}$										
2. Pregnancy test (if applicable)		x^1									x^1	
Transient elastrography	x^3	x^3									x	

CBC – complete blood count; RFT – renal function test; LFT – liver function test; CRP – C-reactive protein; LDL - low-density-lipoprotein; HDL high-density-lipoprotein

[1] Prior to hyperinsulinemic euglycemic clamp

[2] Subject will have lifestyle intervention depending on treatment group assigned

[3] Can be done at either screening or randomization prior to FMT/sham infusion

7. Sample Size

We hypothesize that FMT is superior to lifestyle intervention alone in inducing a sustainable changes of lean-associated microbiota. A previous study observed that FMT led to engraftment of an average of 10% lean-associated microbiota in subjects with metabolic syndrome[12]. Using FMT for the treatment of obesity, however, we consider a higher percentage of sustainable change in microbiota in the majority of recipients is a prerequisite for further research in this field. Therefore, our sample size estimation was based on the assumption that FMT for obesity would achieve at least 20% lean-associated microbiota in 80% of recipients whereas only 20% of patients using lifestyle intervention would achieve this target. A total sample size of 14 evaluable cases per group will be required with a power of 90% to detect this difference at 5% level of significance (Fisher's exact test). Assuming that 30% of patients will lose to follow up, a total of 60 evaluable cases will be recruited to study. Sustainable changes in lean-associated microbiota is defined as lean-associated-microbiota constitutes 20% of recipient's microbiota up to 6 months after FMT or lifestyle intervention.

8. Data Analysis

Metagenomics reads will be quality-filtered using Trimmomatic (v0.39). Host reads will be removed by aligning against human genome hg38 by Kneaddata (v0.7.2). Taxonomic profiling will be performed by aligning reads to MetaPhlAn2 reference database. The resulting taxonomic table will be analyzed in R (v3.5.2). Microbial diversity will be calculated using R packages phyloseq and vegan. Differential features were identified by LEfSe (Linear discriminant analysis Effect Size).

Continuous variables will be expressed in mean (SD) and compared between both treatment groups using unpaired t test. Categorical variables will be compared using χ2 test or Fisher exact test as appropriate. Intention-to-treat analysis will be performed for each outcome. Missing data will be imputed with the use of the last-observation-carried-forward method, whereby missing values will be replaced with the last non-missing value. A two-sided p value of less than 5% will be considered to be statistically significant. Safety data will be summarized with the use of descriptive statistics. All statistical tests were performed with SPSS Statistics version 20 (IBM Corporation, Armonk, NY) or R (v3.5.2).

Screening tests data will be summarized with the use of descriptive statistics. We also aim to compare the fecal microbiota of subjects. Overall diversity will be studied using the Shannon's index and the inverse Simpson's index computed from OTUs. The overall changes in microbiota comparison will be assessed and visualized using principal component analysis. Changes in continuous variables, including the quantities of bacteria, will be compared using t-test or Mann-Whitney's test; whereas changes in categorical variables will be compared using Chi-square test or Fisher's exact test.

9. Purpose and Potential

To the best of our knowledge, no study has prospectively assessed the efficacy and safety of FMT compared with placebo in obese subjects with diabetes. Intestinal microbiota transplantation can potentially represent a novel and effective way to treat obesity with minimal adverse effects. FMT protocols are running in many centers worldwide and the procedure has been endorsed by Food and Drug Administration (FDA) for the management of *Clostridium difficile infections*. Therefore, the procedure can be immediately applied in clinical practice when confirmed to be beneficial in subjects with diabetes and obesity. This will have a big impact for an emerging disease with enormous morbidity and mortality in Hong Kong. To the least, the study results can immediately support the next phase of study.

10. Relevance to Clinical Applications

The infrastructure and support at Prince of Wales Hospital is appropriate for this study. We also have expertise in the assessment and analysis of the intestinal microbiota in various gastrointestinal diseases[10], and our laboratory will be able to support the donor screening test and storage of fecal microbiota. This proposal will not only facilitate the workflow of FMT but also has the potential to provide data on the characteristics of donor and impact of FMT on the intestinal microbiota. The potential gain of subjects is great if they have one more easy and effective alternative therapy. The risk of FMT is minimal and the cost is lower compared with other treatments.

11. Privacy and Confidentiality Protection

Personal identifiers of the subjects, including name, address, identification numbers (e.g. the Hong Kong ID card, passport number, hospital number), electronic contact details (e.g. telephone number, fax number, email address) and non-genetic physical identifiers (e.g. photograph) will be kept confidential and will not be shared with any third party.

12. Data sharing

The data generated in this project are precious and will be useful for the international research community. As such the data (including genetic, metagenomics, metabolomics, microbiota and clinical information) will be removed of the personal identifiers listed above (i.e. de-identified) and will then be deposited in a secure electronic database (e.g. a government-run health research database such as the database of Genotypes and Phenotypes (dbGaP) of the US National Institutes of Health, Sequence Read Archive (SRA) of US National Institutes of Health, China National GeneBank or one administered by an academic institution such as the Li Ka Shing Institute of Health Sciences of The Chinese University of Hong Kong, or the Wellcome Trust Sanger Institute). Access to these data will only be granted to qualified researchers who have the support of their institution or employer. These researchers will be required to agree not to attempt to identify the donor(s) of the genetic information.

13. Potential Risks

Perforation may occur during the OGD, but the complication rate is overall less than 1%.

There is a minimal risk of infectious disease during fecal microbiota transplantation but there is a robust screening for the donor before transplantation. To date, FMT appears to be safe. More than 200 fecal transplantation procedures at the Academic Medical Center in Amsterdam and more than 3,000 at the Centre for Digestive Diseases in Sydney, Australia, without any serious adverse events. Adverse effects of FMT such as bloating, flatulence, borborygmus, vomiting and abdominal discomfort may occur occasionally.

There may be some discomfort or minor bruising from blood taking. Fainting is an occasional adverse event related to blood taking. There is a slight possibility of infection of the skin or vein. Subject may experience some pain and bruising from cannulation during the hyperinsulinemic euglycemic clamp. Subject's blood sugars will be monitored closely after the clamp procedure to ensure it does not go too high or too low.

The de-identified information in the database would only be accessible to qualified researchers. Furthermore, their agreement not to use this information to attempt to identify the donor of the information is a safeguard to protect subjects' confidentiality. However, due to the fact that technologies available in the public domain today, and technological advances expected over the next few years, might make the identification of specific individuals from genetic and other information feasible and increasingly straightforward. There may be potential risks (e.g. those related to insurance, employment, computer security breaches, and law enforcement agencies) to subjects and their family members who may share certain genetic characteristics.

14. No return of research results

The research results from this study will not be returned to subjects. In other words, these data would not be used to influence the clinical care that subjects (or their family members genetically related) would receive. The possible benefits from this research would be the better prenatal care and management in the future.

15. Costs, expenses and payments

In order to help subjects with the costs of taking part in the study, including travel costs and other expenses, an amount of HKD $30-100 will be given to subjects for each required study visit. The amount is dependent on the type of study procedure to be done on the visit.

16. Withdrawal of Consent

Subject can at any time withdraw from this study. All samples collected from the subject may be destroyed upon request. However, de-identified data that have already been deposited to the above mentioned database cannot be removed and data downloaded by qualified researchers mentioned above would not be able to be retrieved. The withdrawal would not in any way affect the medical care that the subject would receive from our hospital.

17. Disclosure

The study will be conducted in compliance with Declaration of Helsinki and ICH-GCP guidelines.

18. Reference List

1. Ma RC, Lin X, Jia W. Causes of type 2 diabetes in China. Lancet Diabetes Endocrinol 2014;2:980-91.
2. Haslam DW, James WP. Obesity. Lancet 2005;366:1197-209.
3. Poobalan AS, Aucott LS, Smith WC, et al. Long-term weight loss effects on all cause mortality in overweight/obese populations. Obes Rev 2007;8:503-13.
4. Dombrowski SU, Knittle K, Avenell A, et al. Long term maintenance of weight loss with non-surgical interventions in obese adults: systematic review and meta-analyses of randomised controlled trials. BMJ 2014;348:g2646.
5. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature 2009;457:480-4.
6. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-31.
7. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341:1241214.
8. Smith MI, Yatsunenko T, Manary MJ, et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 2013;339:548-54.
9. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile. New England Journal of Medicine 2013;368:407-415.
10. Moayyedi P, Surette MG, Kim PT, et al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology 2015;149:102-+.
11. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143:913-916.
12. Li SS, Zhu A, Benes V, et al. Durable coexistence of donor and recipient strains after fecal microbiota transplantation. Science 2016;352:586-9.
13. Kelly CR, Kahn S, Kashyap P, et al. Update on Fecal Microbiota Transplantation 2015: Indications, Methodologies, Mechanisms, and Outlook. Gastroenterology 2015;149:223-37.
14. Kassam Z, Lee CH, Yuan YH, et al. Fecal Microbiota Transplantation for Clostridium difficile Infection: Systematic Review and Meta-Analysis. American Journal of Gastroenterology 2013;108:500-508.