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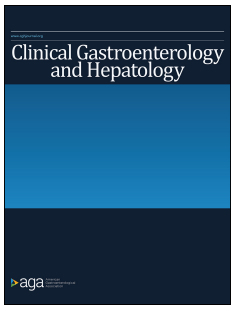

Effect, tolerability, and safety of exclusive palatable elemental diet in patients with intestinal microbial overgrowth

Ali Rezaie, Bianca W. Chang, Juliana de Freitas Germano, Gabriela Leite, Ruchi Mathur, Krystyna Houser, Ava Hosseini, Daniel Brimberry, Mohamad Rashid, Sepideh Mehravar, M.J. Villanueva-Millan, Maritza Sanchez, Stacy Weitsman, Cristina M. Fajardo, Ignacio G. Rivera, Joo Lijin, Yin Chan, Gillian M. Barlow, Pimentel Mark

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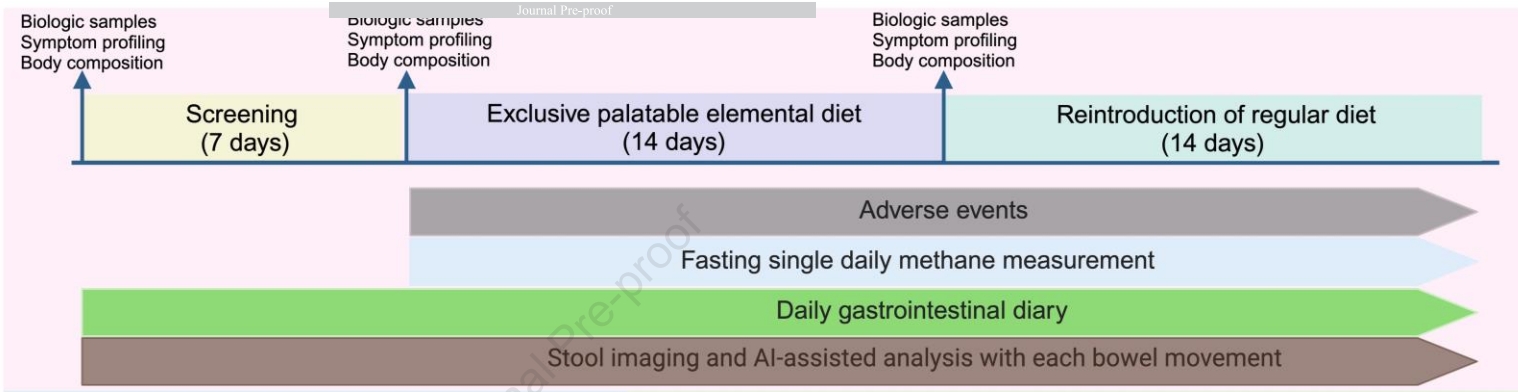

Baseline characteristics

- 30 subjects (63.3% female)
- 20% SIBO, 40% IMO, 40% SIBO/IMO
- 14% treatment naive

Results

- **100% tolerated the exclusive palatable elemental diet**
- **73% normalized their breath test**
- **83% symptom relief based on subjective global assessment (SGA)**
- **Significant decrease in fecal *M. smithii* and *Fusobacterium***
- **No serious/severe adverse events**

Effect, tolerability, and safety of exclusive palatable elemental diet in patients with intestinal microbial overgrowth

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Abstract

Background & Aims: Elemental diets (EDs) have desirable safety and efficacy profiles in several clinical settings partly due to modulation of gut microbiome. Palatability of EDs remains the main barrier to compliance/adherence, and their effect has not been prospectively explored in microbiome-driven disorders such as small intestinal bacterial overgrowth (SIBO) and intestinal methanogen overgrowth (IMO). We aimed to assess the effect, tolerance, and safety of a novel palatable ED (PED) in subjects with IMO and/or SIBO.

Methods: Adult subjects with positive lactulose breath tests (LBT) for SIBO and/or IMO completed one week of screening, 2 weeks of exclusive oral PED, and 2 weeks of follow-up during reintroduction of regular diet. Primary endpoint was changes in stool microbiome after PED and reintroduction of regular diet. Secondary endpoints included tolerability, rate of normalization of LBT, change in stool form based on daily diary and artificial intelligence-analyzed images, symptomatic response, and adverse events.

Results: All 30 enrolled subjects tolerated the PED and completed the trial. Several taxonomic differences were detected including decreased relative abundance of *Prevotella*_9 and *Fusobacterium*. Abundance of *Methanobrevibacter smithii* decreased at the end of the trial and correlated with average daily methane levels ($p=0.024$, $r=0.489$). Maximum methane levels (41±35 to 12±15 ppm, $p<0.001$) and hydrogen rise (43±42 to 12±11 ppm, $p<0.001$) dropped significantly, with 73% normalizing their LBT. Adequate global relief of symptoms was reported in 83% of subjects. No serious or severe adverse events were observed.

Conclusion: PED significantly impacts the gut microbiome. Tolerance to EDs improve with enhanced palatability. Larger studies with longer follow-up are needed to assess response durability. (ClinicalTrials.gov ID: NCT05978973)

Keywords: elemental diet, microbiome, archaea

Introduction

Elemental diets (EDs) are specialized nutritional formulas designed to provide complete or supplemental nutrition in a highly digestible form and contain the required daily allowance of vitamins, major/trace minerals, fat, free amino acids, and carbohydrates. EDs have desirable safety profiles, and their therapeutic benefits have been elucidated in several clinical settings including Crohn's disease (CD), chronic pancreatitis, and eosinophilic esophagitis/gastroenteritis (EoE/EGID).[1] The potential mechanisms of action for ED include their lack of antigenicity which minimizes immune system activation, modulation of the gut microbiome, reduction in pro-inflammatory cytokines, improving intestinal permeability, high absorption index, reduced fecal load, optimal nutritional composition, and lack of food additives/preservatives.[1]

Intestinal microbial overgrowth syndromes are luminal gastrointestinal disorders characterized by abnormal relative and total abundance of microbes such as methanogenic archaea (intestinal methanogen overgrowth, IMO)[2] and small bowel coliform bacteria (small intestinal bacterial overgrowth, SIBO).[3] Effective treatment remains elusive, with antibiotics as the mainstay, but they succeed in only half of SIBO[4] cases and even less in IMO.[5] In addition, high rate of recurrence may necessitate repeated courses of antibiotic therapy. In a prior retrospective study, ED showed promising results in the treatment of SIBO, however, these findings have not been corroborated in prospective trials.[6]

Palatability remains the main barrier to using EDs in clinical practice and organoleptic unacceptability (i.e. poor appearance, smell, taste, aftertaste, and consistency) results in poor compliance both in pediatric and adult populations.[1]

In this prospective, open-label trial, we aimed to assess the effect, tolerability, and safety of an exclusive two-week course of a novel palatable elemental diet (PED) in adult subjects with SIBO and/or IMO.

Methods

Participants

Eligible subjects were symptomatic adults between 18 and 85 years of age with a positive 120-minute lactulose breath test (LBT) for IMO, SIBO, or both (ClinicalTrials.gov ID: NCT05978973). Exclusion criteria included diabetes, pregnancy, breastfeeding, phenylketonuria, inflammatory bowel disease, eosinophilic GI disorder, active infection, or antibiotic use in the last month. Use of concomitant medications were allowed except for antibiotics. The protocol was approved by the Cedars-Sinai institutional review board and all subjects provided written informed consent. All authors had access to the study data and reviewed and approved the final manuscript. Further details are provided in the Supplementary Methods.

Study design

Eligible subjects underwent 1 week of screening, 2 weeks of exclusive oral PED, and 2 weeks of follow-up after returning to their regular diet (Supplemental Figure 1). The primary endpoint was

the change in stool microbiome after PED and regular food reintroduction. Secondary endpoints included tolerance, LBT normalization rate, stool form changes, symptomatic response, and adverse events.

The PED (mBiota Elemental, Good LFE, Santa Monica, USA) was provided in 300-calorie packets, adjusted eucalorically to each participant's caloric needs (Supplemental Figure 2). Additional packets were provided for hunger, with daily consumption documented. Water intake was unrestricted, but other foods were prohibited during the diet phase. After completing the diet, participants transitioned to bland foods (e.g., rice, potatoes, eggs, chicken, beef) for 2–3 days before resuming their regular diet.

Visits and data collection

Subjects were seen in person at baseline and weeks 1, 3, and 5, with telephone visits at weeks 2 and 4 (Supplemental Figure 1). At baseline, subjects underwent a physical examination and completed a medical history questionnaire and modified structured assessment of gastrointestinal symptoms (SAGIS) questionnaire in which British English was adapted to American English.[7] Brain fog score was the sum of five binary questions on confusion, cloudiness, impaired judgment, poor short-term memory, and difficulty concentrating.[8] Constipation score was derived from three SAGIS Likert scale items (0–4) assessing pain/discomfort before defecation, difficulty defecating, and reduced bowel movement frequency with hard or lumpy stools. Daily, subjects completed a gastrointestinal diary which recorded symptoms using a visual analog scale (VAS) on a scale of 0 (no symptom) to 100 (maximum symptom).[9] Subjects were asked to record their stool images

for the entire duration of the trial using a validated HIPAA-compliant artificial intelligence-based smartphone application (Dieta AI stool tracker, Dieta health, USA) which objectively assesses stool characteristics.[10] Subjects also documented the time spent in the bathroom for each bowel movement.

After completion of the PED, subjects underwent a physical examination and completed modified SAGIS questionnaires[7] and symptom VAS. At the end of the trial, modified subjective global assessment (SGA)[11], and modified SAGIS questionnaires were completed. (Supplemental Methods)

Biologic and anthropometric testing

Fasting body composition assessments via bioelectrical impedance (InBody970, Cerritos, CA) and peripheral blood samples were taken at screening, after PED, and at the end of the trial. Levels of glucose and electrolytes were determined (COBAS, Roche Diagnostics). Stool samples were collected at screening, after PED, and at the end of the trial for 16S rRNA gene sequencing. Presence of fecal *Methanobrevibacter smithii (M. smithii)* was further assessed by Digital Polymerase Chain Reaction (dPCR). (Supplemental Methods) LBTs were performed at baseline and after PED using a BreathTracker SC (Quintron Instrument Company, Milwaukee, WI). Abnormal BTs were classified as SIBO (Peak H_2 rise of ≥20 ppm within 90 minutes); IMO (CH_4 of ≥10 ppm at any timepoint); IMO/SIBO (meeting both SIBO and IMO criteria described above), flatline test (nonmethane fixed-hydrogen producers), or elevated baseline (H_2 ≥ 20 ppm at baseline).[12-14] LBT was considered normal if none of the aforementioned criteria were met.[12] Upon

consumption of the PED to the end of the study, subjects collected single fasting breath samples at home every morning using a specialized test tube (Extainer, Labco, Ceredigion, UK) for determination of fasting single daily methane measurements (SMM). The response in patients with IMO, as determined by SMM, was defined as achieving a level ≤5 on the final day of the trial.[5]

Statistical analysis

The effects of PED on symptoms were estimated from daily diaries and SAGIS as score differences between the reintroduction and screening phases, analyzed using paired T-tests. BMs were summarized with an alluvial plot by the most frequent BSS type of the week per patient. For electrolyte and glucose levels, following testing for normal distribution, one-way ANOVA was performed to assess overall differences in measurements across the three blood draw visits. A p-value of 0.05 or less was considered statistically significant. Bioinformatic analyses are detailed in the Supplementary Methods.

Results

From August to December 2023, 33 subjects were screened and 30 fulfilled the inclusion/exclusion criteria and were enrolled (63% women, median (range) age 45 (23-73) years). Of these, 40% had IMO, 20% SIBO, and 40% IMO/SIBO. (Table 1) No subject had a flatline or elevated baseline pattern. All subjects (100%) completed the 2-week course of exclusive PED.

Changes in stool microbiome

A total of 84 stool samples (29 from the baseline, 27 after completing the PED, and 27 at the end of the trial) were collected from 30 subjects and underwent 16S rRNA gene sequencing. As compared to baseline, alpha diversity decreased after the PED but was not statistically different at the end of the trial. (Figure 1a) Similarly, pairwise beta diversity changed after the PED (Bonferroni p-value < 0.001) but was not statistically different from the baseline at the end of the trial (Bonferroni p-value = 1.000). (Figure 1b-1d)

After the PED, 30 bacterial families showed significant differences in relative abundance (RA) (Supplemental Figure 3a) along with 70 bacterial genera. (Supplemental Figure 3b) The most significant change was a decrease in RA of *Prevotella_9* (Log2 fold change (Log2FC)=-11.6, q value<0.0001).

At the end of the trial, *Fusobacteriaceae* was the only family with a significant decrease in RA compared to baseline (Log2FC=-2.21, q value=0.002). Five genera showed significant RA changes from baseline. *Prevotella_9* genus showed a further reduction at the end of the trial (Log2FC=-12.1, q value<0.0001). Moreover, *Fusobacterium* and *[Eubacterium] fissicatena* group showed a decrease in RA from baseline (Log2FC=-2.26, q value=0.002 and Log2FC=-3.57, q value<0.0001, respectively). *Enterobacter* RA, which showed a decrease in RA after the PED (Log2FC=-2.51, q value<0.0001), increased at the end of the trial (Log2FC=3.14, q value=0.002). *Lachnospiraceae* UCG-001 showed a significant decrease in RA after the PED (Log2FC=-6.22. q value<0.0001) and at the end of the trial (Log2FC=-2.80, q value=0.035). (Figure 2a-2c)

Changes in symptoms and stool form

All subjects completed the questionnaires including daily queries, but 2 opted out of stool image documentation, resulting in 1,401 images from 28 participants. Average weekly completeness of the bowel movements was 38% during screening week which significantly increased to 68% while on PED and 69% at the final week of the trial (p=0.03). (Supplementary Figure 4a) As expected, the overall number of bowel movements decreased during PED while the looseness of the stool increased. (Supplemental figure 4b) Percentage of bowel movements with normal consistency (i.e. BSS 3-5) increased from 56% at baseline to 71% at the end of the trial but did not reach statistical significance (p= 0.35).

As compared to the baseline, weekly average of daily symptom severity during the second week of PED showed significant improvement in abdominal discomfort (p= 0.02), bloating (p<0.001), distention (p<0.001), constipation (p=0.04), and flatulence (p<0.001). No significant change was observed for brain fog (p=0.63), belching (p=0.80), abdominal pain (p=0.38), bowel movement duration (p=0.27), urgency (0.35) and fatigue (0.59), while diarrhea severity increased congruent with stool image AI analysis (p=0.001). During the final week of the trial with reintroduction of regular diet, significant improvement was observed in abdominal pain (p=0.001), discomfort (p<0.001), bloating (p<0.001), distention (p<0.001), diarrhea (p=0.001), constipation (p=0.003), fatigue (p=0.001), urgency (p=0.02), flatulence (p=0.006), and brain fog (p=0.004). No significant improvement was observed in belching (p=0.23) and duration of bowel movements (p=0.32)

(Table 2). At the end of the trial, adequate global relief of symptoms was reported in 25 out of 30 (83%) subjects.

Changes in exhaled hydrogen and methane

Repeat LBT following completion of the PED normalized in 22 (73.3%) subjects. Normalization rates were 58%, 100% and 75% in subjects with IMO, SIBO and IMO/SIBO, respectively. Irrespective of normalization of LBT, subjects showed a significant drop in maximum exhaled methane levels (41±35 to 12±15 ppm, $p<0.001$) and peak rise in hydrogen within 90 min (43±42 to 12±11 ppm, $p<0.001$). (Table 3) Following PED, a significant reduction in hydrogen levels was observed at the 60-, 75-, 90-, 105-, and 120-minute timepoints during the LBT suggesting a shift in hydrogen production from a small bowel pattern (rise of hydrogen ≥20ppm within 90 minutes) to a delayed rise consistent with a colonic fermentation pattern (Figure 3a). After PED, significant reductions in methane levels were seen at all time points of the LBT (Figure 3a). Following PED, the baseline area under the curve for hydrogen (up to 90 minutes) significantly decreased from 136±122 to 68±78 ppm ($p=0.001$), and methane (up to 120 minutes) decreased from 258±212 to 72±93 ppm ($p<0.001$).

Average daily SMMs in 24 subjects with IMO or IMO/SIBO were significantly lower during the PED (4.0±5.7 ppm, $p<0.001$) and follow up periods (4.5±5.8 ppm, $p=0.042$) compared to baseline (14.8±19.6 ppm) (Figure 3b and supplemental Figure 5a). Average SMMs in these subjects dropped below 5 ppm (4.45±6.69ppm) by the fifth day of PED and 18 (75%) had an SMM≤5 by the last day of the trial.

Of 24 subjects with IMO or IMO/SIBO, 23 stool samples were available at baseline and 14 (61%) were positive for *M. smithii*, the most abundant methanogenic archaeon in human. Fecal abundance of *M. smithii* decreased after PED (p=0.206, n=11; Supplemental figure 5b) and reduction reached statistical significance by the end of the trial, when 92% of the subjects demonstrating either decreased or undetectable levels of *M. smithii* (p=0.017, n=13; Supplemental Figure 5b). Importantly, subjects with no detectable DNA copies of *M. smithii* at baseline did not show a significant change in *M. smithii* levels at the end of the trial (n=9, p=0.500), further supporting the reductive effect of the PED on *M. smithii.* Fecal abundance of *M smithii* at baseline, post-PED, and at trial completion positively correlated with SMM at baseline (r=0.627, p=0.001, n=23), average SMMs during the PED phase (r=0.581, p=0.007, n = 20), and average SMMs during the regular diet reintroduction phase (r=0.489, p=0.024, n=21). (Supplemental Figure 5c)

Body composition and safety profile

As compared to baseline, after the PED, and after reintroduction of regular diet subjects significant drops were observed in weight (70.4±20.0 Kg vs. 67.0±19.2 Kg vs. 67.7±19.8, P<0.01), total body fat (20.4±13.6 Kg vs. 19.0±13.0 Kg vs. 18.7±13.2 Kg, P<0.01), and visceral fat area (42.8±25.9 cm^2 vs. 39.9±26.8 cm^2 vs. 39.5±27.2 cm^2, P<0.01).

Following PED and at the end of the trial, fasting morning levels of sodium, potassium, chloride, and bicarbonate were within normal limits and fasting blood glucose levels did not show hyper- or hypoglycemia. (Supplementary Figure 6) No serious or severe adverse events were reported.

Mild adverse events were reported in 11 subjects including diarrhea (n=3), cramps (n=2), nausea (n=4), heartburn (n=2), fatigue (n=3), urgency (n=1), belching (n=1), transient hemorrhoidal bleeding (n=2). Adverse events did not lead to study withdrawal. None of the subjects required antimicrobial therapy during the study.

Discussion

This study is the first prospective trial examining the use of an elemental diet in IMO/SIBO, providing valuable insights into the pathophysiology of these conditions. PED modulated the stool microbiome and improved clinical symptoms and hydrogen/methane production, emphasizing the crucial role of diet in IMO/SIBO.

Several clinically significant microbiome changes were observed at the family and genus level. As compared to baseline, at the end of the trial RA of *Fusobacteriaceae* family and *Fusobacterium genus* significantly decreased. *Fusobacterium* bacteria, known producers of intraluminal hydrogen sulfide, have been implicated in diarrhea, urgency, and gut inflammation.[15, 16] Reduction in the RA of *Prevotella_9* genus is also noteworthy, as *Prevotella_9 copri* correlates with increased abdominal pain in irritable bowel syndrome.[17] The fecal abundance of *M. smithii,* is associated with constipation, bloating and exhaled methane[5], decreased in 92% of subjects who had detectable levels at baseline. Whole-genome metagenomic sequencing is warranted to explore taxonomic, functional, and microbial interaction pathways.

The mechanisms by which PED influences the microbiome are likely multifaceted. 1) PED provides all essential and non-essential amino acids but lacks polypeptides and dipeptides,

making it free of allergens. EDs have shown to modulate the microbiome in food-antigen driven diseases (e.g. EoE/EGID).[1] Second, EDs require minimal digestion, reducing fecal mass, a characteristic associated with their effectiveness in CD and altering the microbiome.[18] Third, PED is a very low-fat diet, with only 6% of total calories derived from fat, consisting exclusively of medium-chain triglycerides (MCTs) and no pro-inflammatory long-chain triacylglycerols (LCTs).[19] Positive effects of low-fat diets on microbiome have been shown in animal models and humans.[20] Fourth, pancreaticobiliary secretions may have modulatory effect on gut microbiome[21] and EDs are weaker stimuli of pancreatic secretions compared to a regular diet.[22] Fifth, free amino acids (e.g. glutamine) have significance in enhancing cellular growth, preventing epithelial atrophy, improving immune response, and strengthening intestinal barrier function and potentially modulate the gut microbiome.[23] Finally, PED's absence of artificial additives avoids potential microbiome-disrupting effects seen with sweeteners and nanoparticles.[24] The magnitude of the effect of a short-term diet free of food additives, as in our study, remains uncertain. Future studies should explore these mechanisms further.

Palatability remains a critical barrier to the use of EDs, with prior studies reporting intolerance rates as high as 41%.[1] In this study, the tolerance rate was 100%, likely due to the organoleptic acceptability of PED, including its appearance, smell, taste, aftertaste, and consistency.[25] These findings underscore the importance of palatability in improving adherence to elemental diets in adult patients.

PED's effects on normalizing 73% of LBTs and reducing exhaled gases were notable. Even among those without full normalization, substantial reductions in exhaled methane and hydrogen levels were observed, indicating significant physiological impacts. The reduction in methane and hydrogen levels along with a shift from small bowel to colonic fermentation patterns further support the hypothesis that PED modulates gut microbes.

The participant cohort emulated the prior description of patients with IMO and SIBO.[2, 26] The majority of the subjects had previously tried several treatments with suboptimal response. Subjects reported significant reductions in abdominal discomfort, bloating, distention, flatulence, and constipation during PED, with further improvements in abdominal pain, diarrhea, urgency, fatigue, and brain fog after reintroducing a regular diet. Expected increased stool looseness during PED, resolved upon reintroducing regular foods. movements[27]. Despite looser stools, bowel movement frequency decreased during PED, consistent with the documented effect of EDs on reducing fecal load.[1] Larger controlled trials are needed to assess the extent of PED effect on symptoms; however, significant challenges exist in designing a controlled dietary trial.

Although EDs are considered nutritionally complete, weight loss has been associated with their use, typically attributed to poor adherence.[28] In this study, weight loss occurred despite optimal compliance and eucaloric design, primarily due to fat loss. The low-fat content of PED may explain this phenomenon, with reductions in total and visceral fat persisting after reintroducing a regular diet. Future studies should evaluate ED's effects on fat metabolism, micro/macronutrient status,

and energy expenditure. Furthermore, due to the restrictive nature of EDs, their potential role in triggering or unmasking underlying eating disorders warrants further examination.

Mild adverse events were reported during the study, including diarrhea, cramps, nausea, heartburn, fatigue, urgency, belching, and transient hemorrhoidal bleeding. Although none of these events led to discontinuation of the formula, they highlight the importance of thoroughly counselling patients about the potential side effects associated with EDs. Preparing patients for these possible reactions can help manage expectations and improve adherence to the dietary regimen.

This study has limitations. Microbial gene functional analysis and microbial pathways/interactions were not performed as these require whole-genome shotgun metagenomic sequencing. Lack of a blinded placebo group with a sham elemental diet is another limitation of our study. While including such a group would have allowed for a more precise assessment of any placebo effect, we determined that challenges of engineering and unknown effects of a similar-tasting non-elemental sham formula combined with possibility of exacerbating patients' symptoms posed an undue burden. The follow-up period after reintroducing regular diet was 2 weeks. A longer follow-up is necessary to evaluate the durability of the observed effects of PED on the microbiome and symptoms.

In conclusion, this trial demonstrates the benefit, tolerance, and safety of PED in IMO/SIBO. PED improved symptoms, reduced microbial gas production, and modulated the microbiome, including reductions in key taxa like *M. smithii* and *Fusobacterium*. High adherence underscores the

297 importance of palatability in EDs. These findings support PED as a promising dietary intervention
298 and highlight the need for controlled trials to explore long-term benefits and mechanisms.

299

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304 Conflict of interest:

305 MP is a consultant for Ferring Pharmaceuticals, Inc, Salvo Health, Dieta Health, and Vivante
306 Health, Inc has received grant support from Bausch Health, and has equity in Gemelli Biotech,
307 Dieta Health, Salvo Health, Vivante Health, and Good LFE. RM has equity in Gemelli Biotech and
308 Good LFE. AR is a consultant/speaker for Bausch Health and has equity in Dieta health, Gemelli
309 Biotech and Good LFE. KH has equity in Good LFE. Cedars-Sinai has licensing agreements with
310 Hobbs Medical and Gemelli Biotech.

311 Authorship Contributions

312 AR (Conceptualization; Formal analysis ; Funding acquisition; Investigation; Methodology; Project
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337

338

Table 1. Demographic and clinical features of the participants

Table 2. Weekly average of daily symptom severity during the screening week, second week of the palatable elemental diet (PED), and the last week of the trial. Brain fog score was derived from five binary questions on confusion, cloudiness, impaired judgment, poor short-term memory, and difficulty concentrating. Constipation score was the sum of three scales assessing pain/discomfort before defecation, difficulty defecating, and constipation.

Table 3. Changes in gas production during breath testing at screening and after palatable elemental (PED) are reported as mean ± standard deviation [median].

Figure 1. a) Shannon entropy (α diversity) at baseline, after palatable elemental diet (PED), and following the reintroduction of regular diet. b) Principal Coordinate Analysis (PCoA) showing β-diversity distribution at baseline (green), after PED (blue), and following the reintroduction of regular diet (pink).

Figure 2. a) Bacterial relative abundance (RA) at the family level from baseline, after palatable elemental diet (PED), and at trial's end. b) Bacterial RA at the genus level from baseline, after the PED, and at trial's end. c) Bacterial genera with significant differences in RA at the end of the trial.

Figure 3. a) Lactulose breath tests before and after palatable elemental diet (PED) show significant drops in hydrogen (All timepoints after 45 minutes) and methane production (all timepoints. b) Reduction in the average daily single fasting methane measurements in patients with IMO or IMO/SIBO throughout 28 days of the PED and reintroduction phases (Green line).

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Characteristic	Value
Demographics	
Female % (n)	63.3 (19)
Age	46.0±14.5
Body mass index	25.4±6.0
Racial and ethnic category % (n)	
African American	10.0 (3)
Caucasian	66.7 (20)
Middle eastern	13.3 (4)
Asian	10 (3)
Symptoms at screening % (n)	
Bloating	100 (30)
Abdominal distention	93.3 (28)
Abdominal pain	66.7 (20)
Abdominal discomfort	73.3 (22)
Diarrhea	63.3 (19)
Constipation	66.7 (20)
Belching	63.3 (19)
Flatulence	100 (30)
Rectal urgency	66.7 (20)
Fatigue	100 (30)
Treatments before PED	83.3 (26)
Antibiotics	83.3 (26)
Elemental diet	26.7 (8)
Promotility medications	63.3 (19)
Complementary and alternative medicine	66.7 (20)
Microbial overgrowth status at baseline % (n)	
IMO	40 (12)
IMO and SIBO	40 (12)
SIBO	20 (6)

PED, palatable elemental diet; IMO, intestinal methanogen overgrowth; SIBO, Small intestinal bacterial overgrowth.

	Mean Screening phase (95% CI)	Mean PED phase (95% CI)	Mean Reintroduction phase (95% CI)	Mean Change from Screening to PED phase (95% CI)	Median % Change (IQR)	P value (FDR Adjusted)	Mean Change from Screening to Reintroduction phase (95% CI)	Median % Change (IQR)	P value (FDR Adjusted)
Pain	29.82 (21.43, 38.21)	25.76 (17.52, 34.01)	18.24 (11.74, 24.74)	-4.06 (-12.37, 4.26)	-11 % (-49, 19)	0.327 (0.479)	-11.58 (-18.05, -5.11)	-41 % (-73, -3)	0.001 (0.002)
Discomfort	41.73 (33.53, 49.94)	31.66 (23.45, 39.87)	23.72 (17.00, 30.45)	-10.07 (-18.43, -1.72)	-30 % (-49, -9)	0.020 (0.05)	-18.01 (-24.74, -11.28)	-39 % (-61, -26)	<0.001 (<0.001)
Bloating	49.79 (41.71, 57.88)	28.71 (20.38, 37.05)	27.45 (19.97, 34.93)	-21.08 (-28.62, -13.55)	-43 % (-75, -15)	<0.001 (<0.001)	-22.34 (-30.05, -14.63)	-40 % (-81, -24)	<0.001 (<0.001)
Distention	50.53 (42.24, 58.82)	27.85 (19.83, 35.88)	28.07 (20.77, 35.37)	-22.68 (-30.45, -14.91)	-40 % (-66, -15)	<0.001 (<0.001)	-22.46 (-30.28, -14.65)	-37 % (-63, -19)	<0.001 (<0.001)
Diarrhea	19.30 (11.87, 26.72)	41.23 (30.15, 52.31)	6.79 (1.10, 12.49)	21.93 (9.64, 34.23)	135 % (-17, 645)	0.001 (0.003)	-12.50 (-19.56, -5.44)	-82 % (-99, -37)	0.001 (0.002)
Belching	24.70 (16.55, 32.84)	23.65 (16.67, 30.62)	19.70 (12.75, 26.64)	-1.05 (-9.33, 7.24)	-6 % (-49, 76)	0.798 (0.801)	-5.00 (-13.35, 3.35)	-18 % (-75, 27)	0.231 (0.252)
Flatulence	38.02 (31.47, 44.57)	20.72 (15.44, 26.00)	25.76 (18.77, 32.74)	-17.30 (-24.28, -10.31)	-42 % (-69, -20)	<0.001 (<0.001)	-12.26 (-20.66, -3.86)	-34 % (-60, -16)	0.006 (0.008)
Fatigue	44.12 (35.31, 52.93)	46.75 (37.31, 56.19)	28.48 (19.91, 37.04)	2.62 (-7.23, 12.48)	-4 % (-21, 66)	0.590 (0.708)	-15.65 (-24.06, -7.23)	-34 % (-75, -5)	0.001 (0.002)
Urgency	32.14 (22.51, 41.77)	36.88 (25.51, 48.26)	21.39 (13.33, 29.44)	4.74 (-5.46, 14.95)	0 % (-54, 78)	0.350 (0.479)	-10.76 (-19.70, -1.81)	-38 % (-85, -7)	0.020 (0.024)
Duration (min)	2.00 (1.15, 2.84)	1.57 (0.90, 2.24)	1.68 (1.06, 2.30)	-0.42 (-1.19, 0.35)	-39% (-100, 18)	0.271 (0.479)	-0.32 (-0.96, 0.33)	-11 % (-72, 54)	0.324 (0.370)
Constipation	4.33 (3.30, 5.36)	3.10 (2.19, 4.01)	2.83 (2.08, 3.59)	-1.23 (-2.38, -0.09)	-25 % (-58, 10)	0.036 (0.072)	-1.50 (-2.45, -0.55)	-50 % (-58, 0)	0.003 (0.005)
Brain Fog	2.00 (1.39, 2.61)	1.80 (1.18, 2.42)	1.07 (0.47, 1.66)	-0.20 (-1.03, 0.63)	-50 % (-90, 0)	0.625 (0.801)	-0.93 (-1.54, -0.33)	-68 % (-100, 0)	0.004 (0.008)

FDR adjusted, false discovery rate-adjusted P value; IQR, interquartile range

	All Subjects (n=30)	IMO (n=12)	Hydrogen SIBO (n=6)	IMO/SIBO (n=12)
Screening maximum CH_4 (ppm)	40.8±35.0 [27.0]	59.8±41.5 [46.0]	7.8±1.0 [8.0]	38.3±22.8 [28.0]
Maximum CH_4, after PED (ppm)	12.4±15.0 [7.0]**	17.1±15.8 [9.0]**	4.0 ±2.3 [4.0]*	12.3 ± 16.9 [7.0]*
Screening baseline CH_4 (ppm)	27.5±25.3 [23.0]	41.1±31.6 [28.0]	3.8±2.8 [3.0]	25.7±13.3 [23.0]
Baseline CH_4 after PED (ppm)	5.2±7.7 [1.0]**	9.0 ± 8.4 [5.0]**	0.8±0.8 [1.0]	3.6±7.7 [1.0]**
Screening peak H_2 rise by 90 min (ppm)	42.8±42.5 [32.0]	6.4 ± 5.1 [5.0]	81.8±45.0 [87.5]	59.7 ± 35.0 [43.0]
Peak H_2 rise by 90 min after PED (ppm)	11.8±11.2 [10.0] **	12.7 ±13.5 [9.0]	11±3.5 [11.0] *	11.3 ± 11.9[8.0] **

** P<0.01; * P<0.05; IMO, intestinal methanogen overgrowth; SIBO, small intestinal bacterial overgrowth; ppm, part per million; PED, palatable elemental diet

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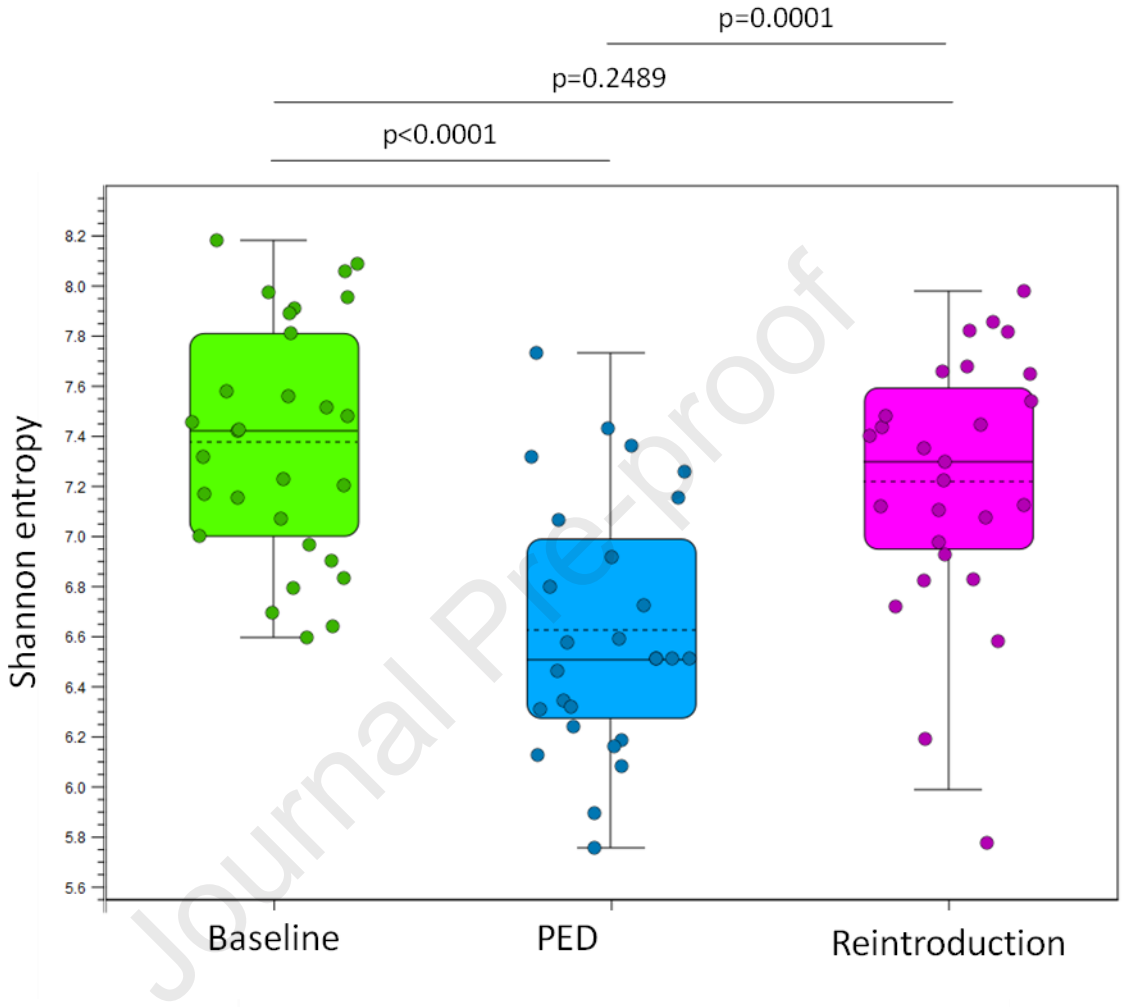

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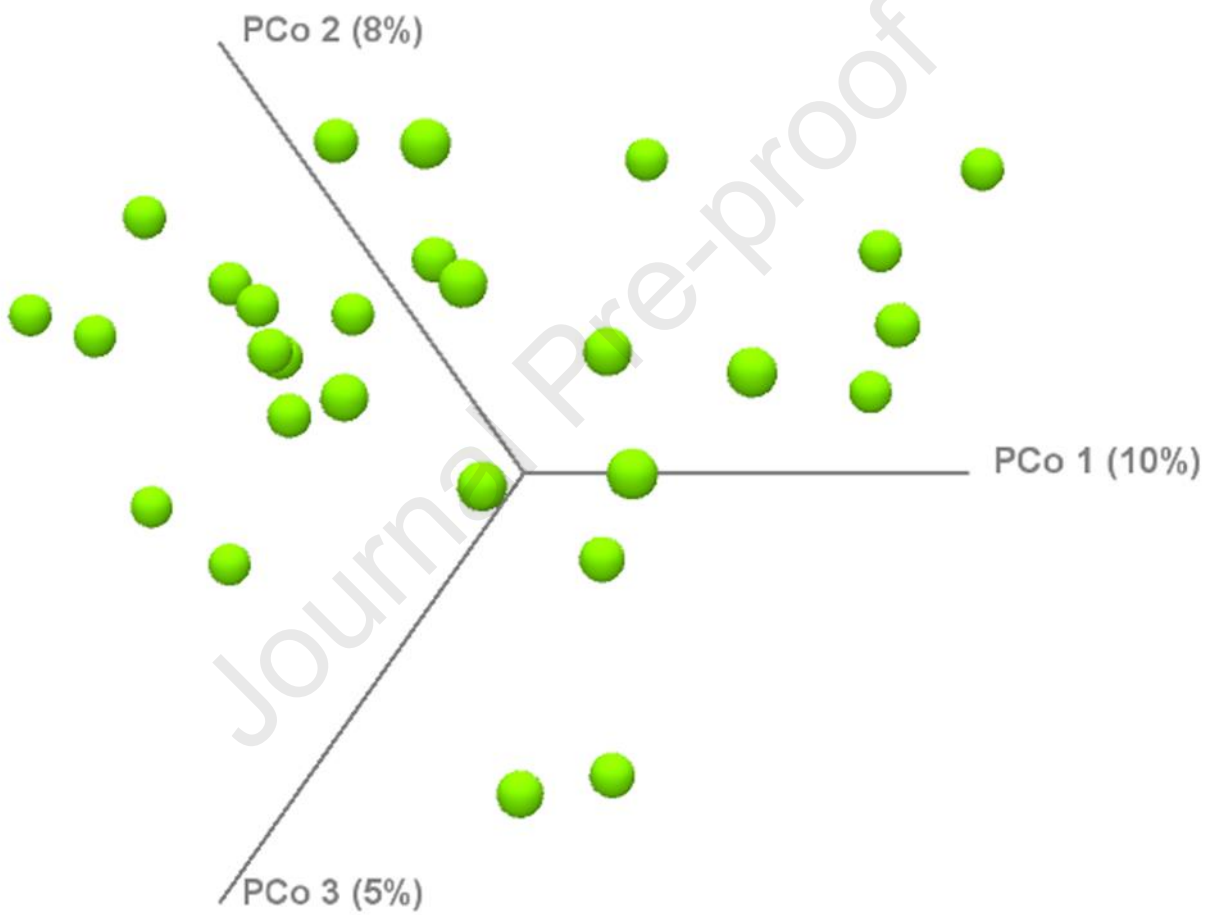

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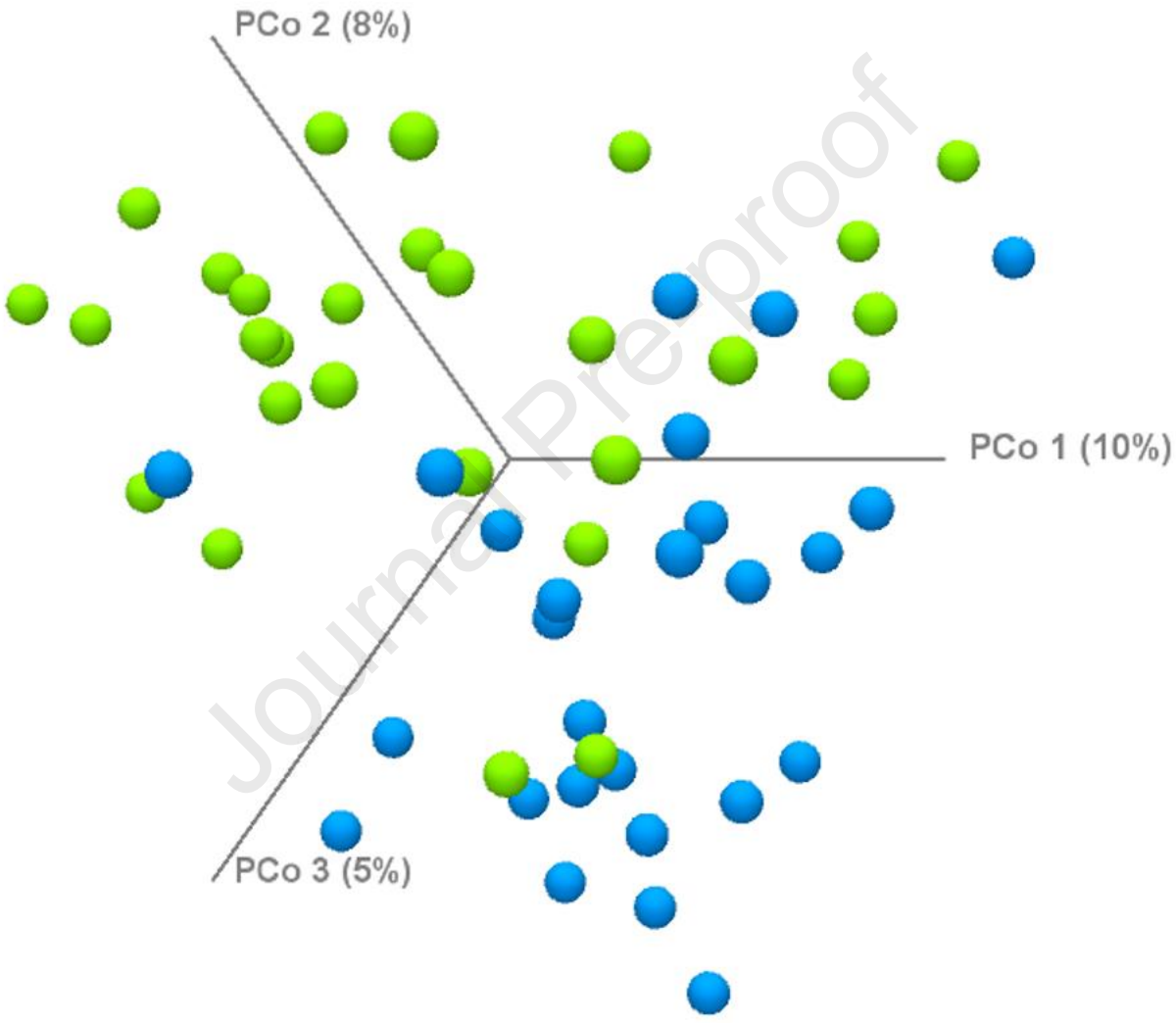

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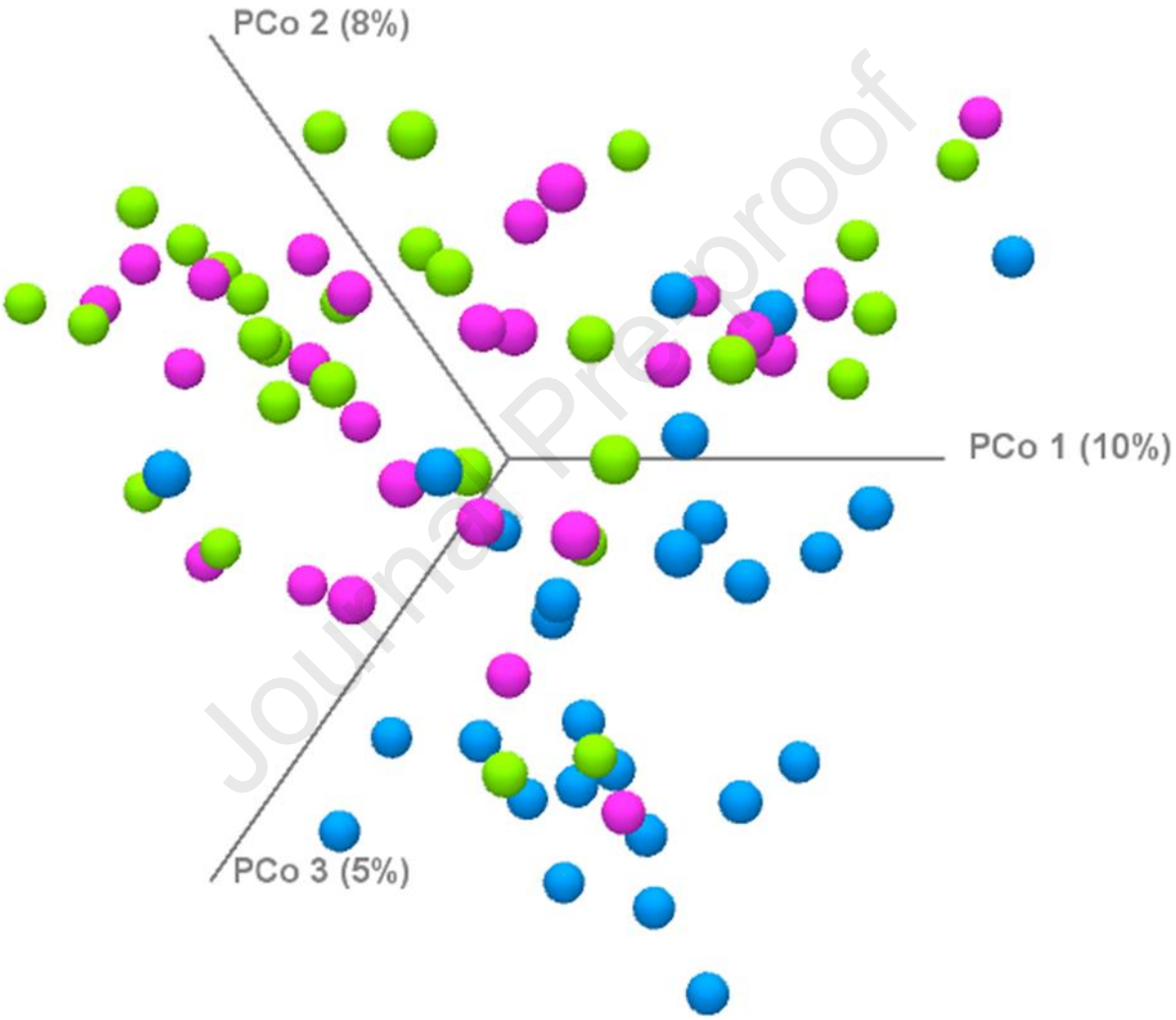

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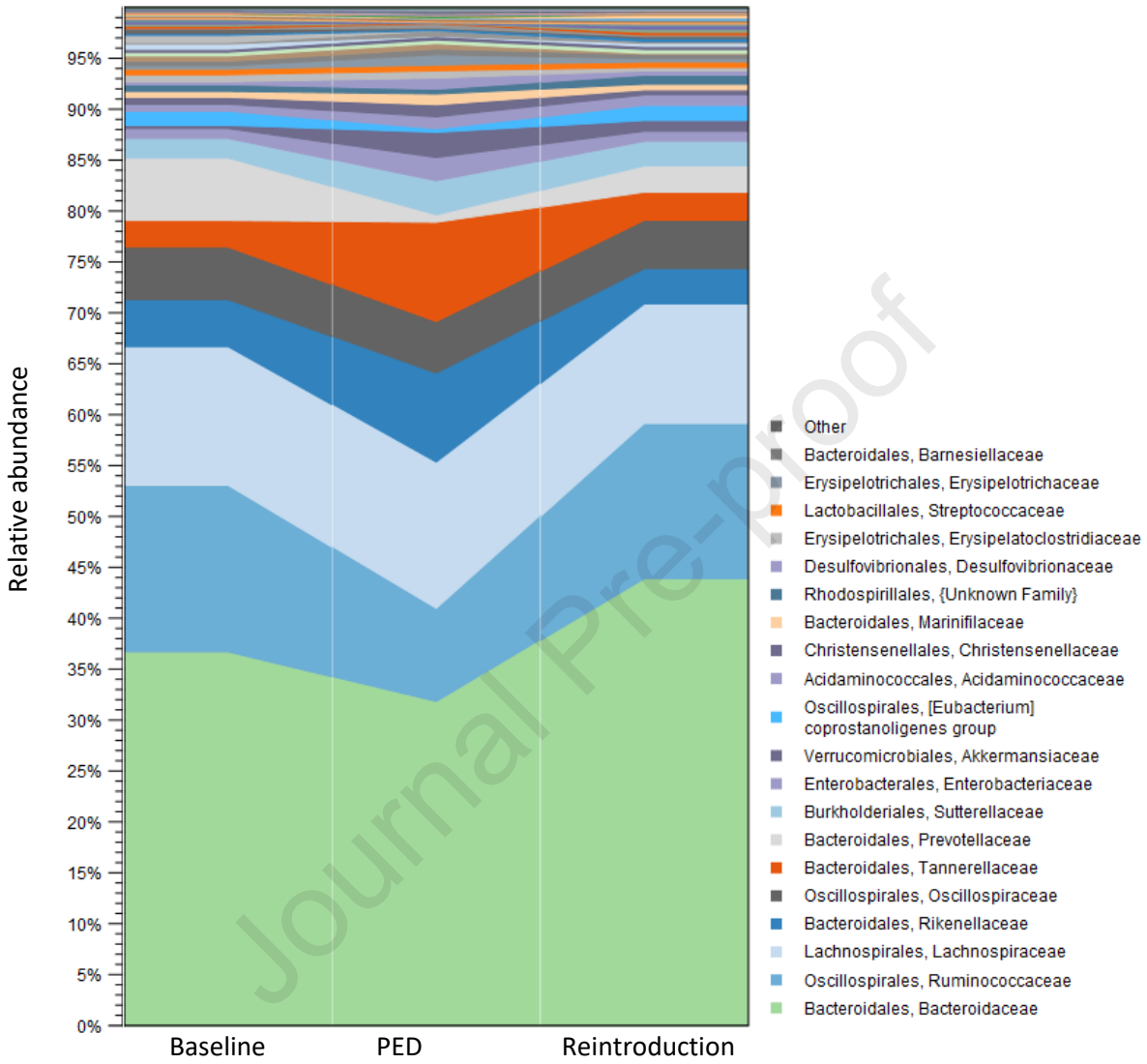

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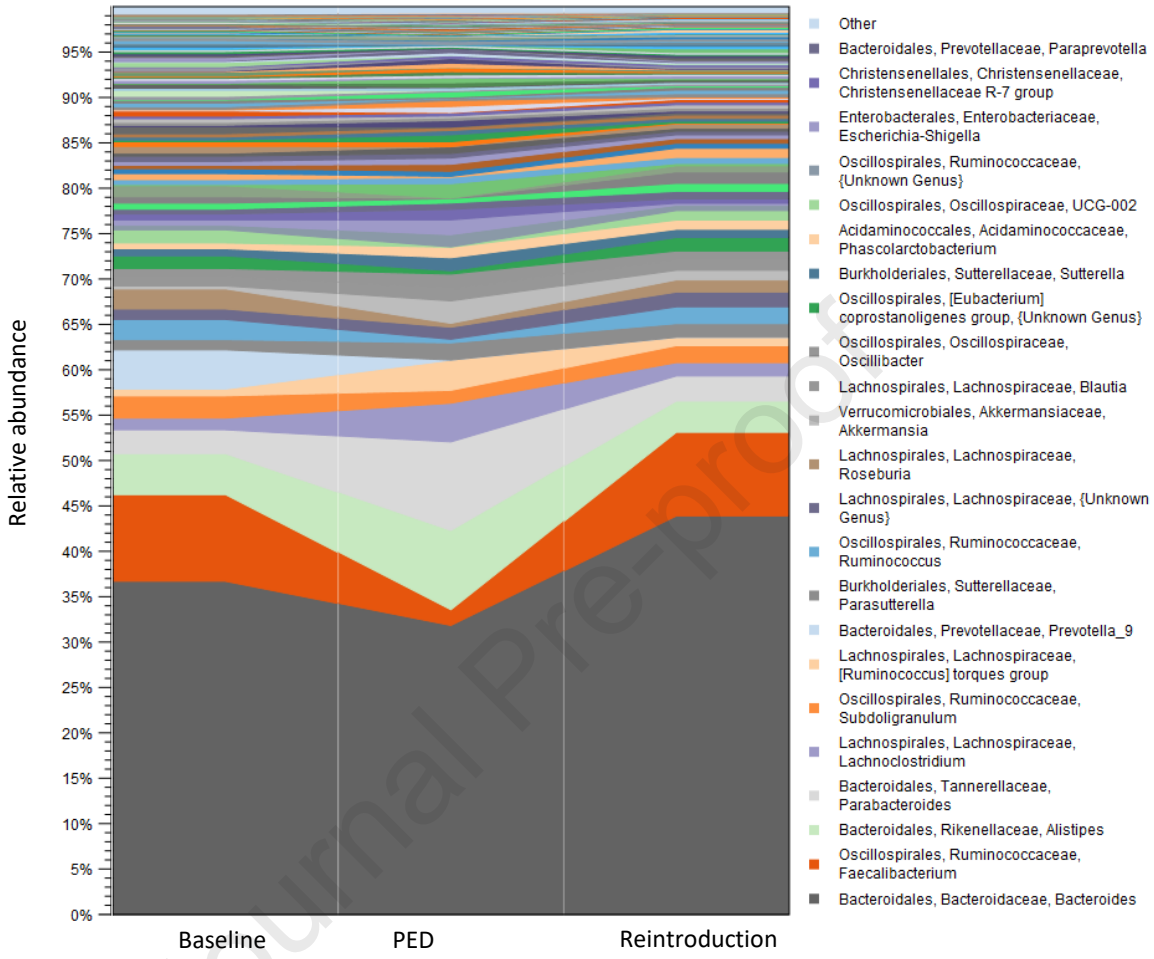

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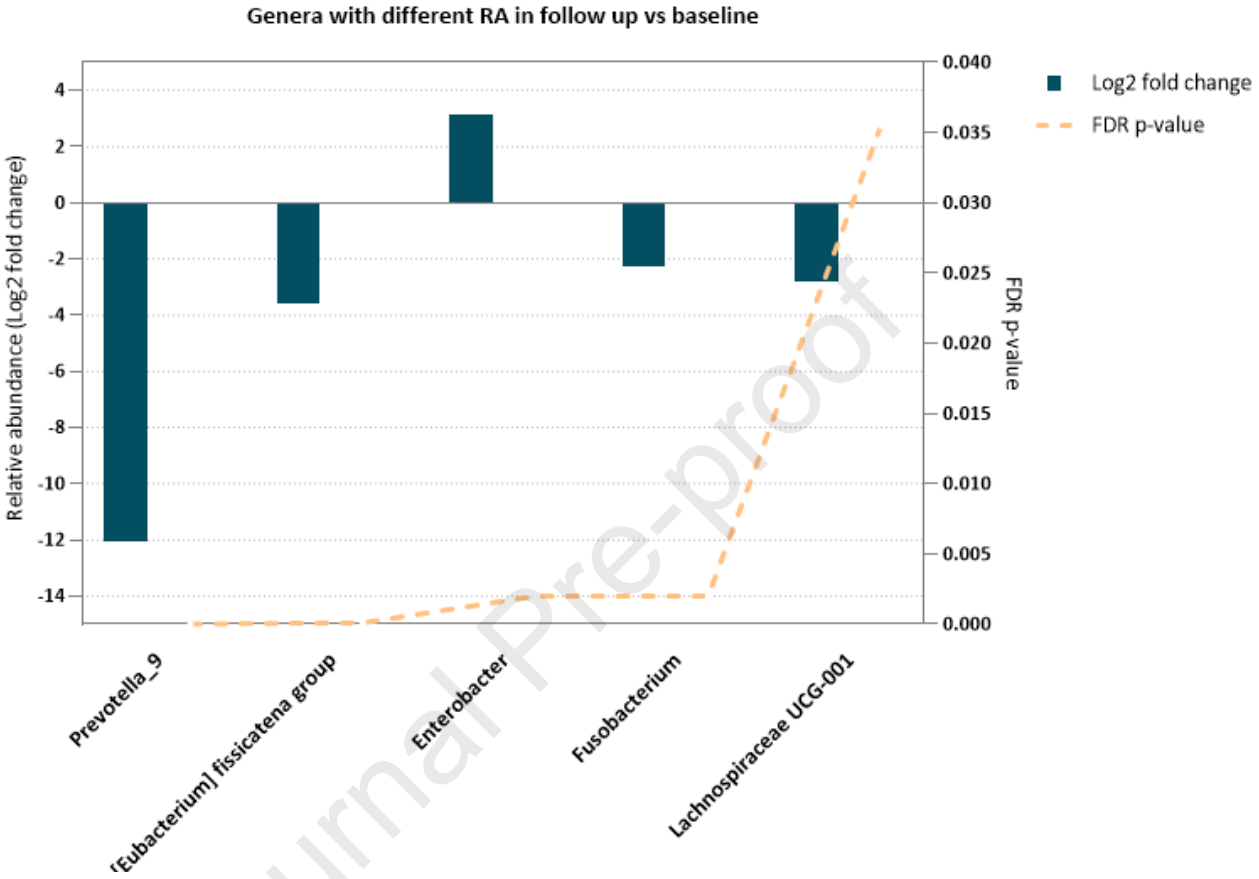

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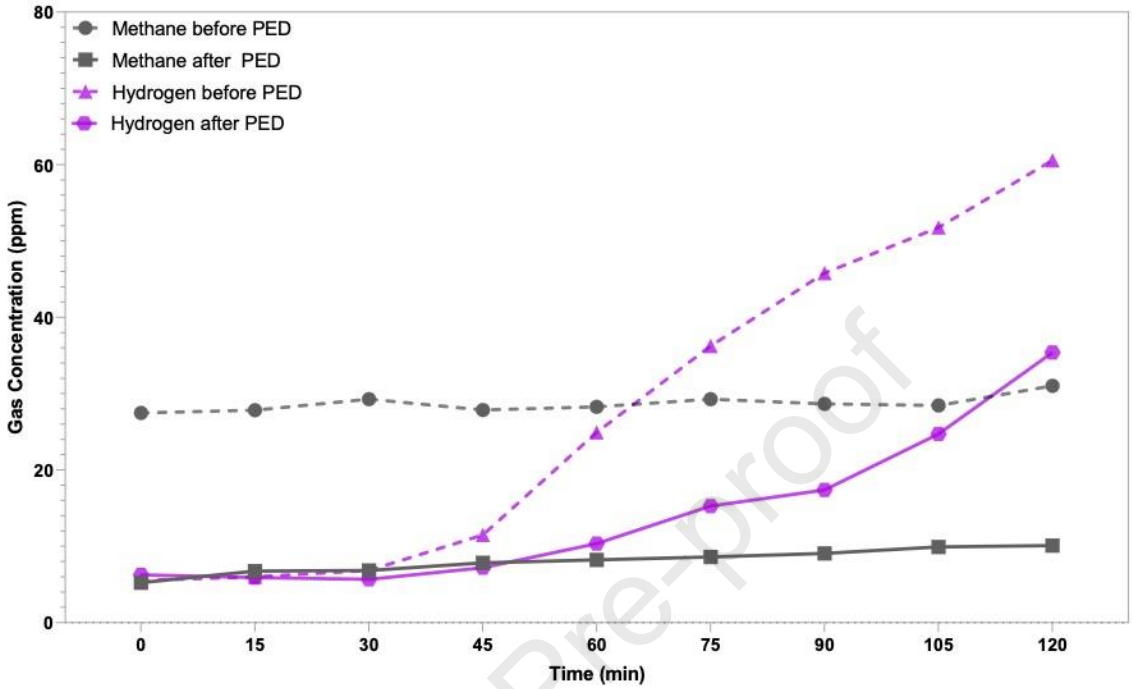

Time (min)	0	15	30	45	60	75	90	105	120
Mean change in CH_4 after PED (ppm)	-22.2 +24.5	-21.07 + 22.0	-22.42 + 21.4	-20.48 + 19.7	-20.03 + 20.7	-20.67 + 24.2	-19.6 + 23.6	-18.53 + 21.2	-20.93 + 28.0
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
Mean change in H_2 after PED (ppm)	0.7 + 0.7	-0.1 +10.3	-1.16 + 9.1	-4.3 +15.7	-14.5 +30.9	-21 +41.7	-28.4 + 45.0	-27.03+51.65	-25.17+ 55.17
p value	0.74	0.96	0.48	0.14	0.01	0.009	0.0017	0.0076	0.01

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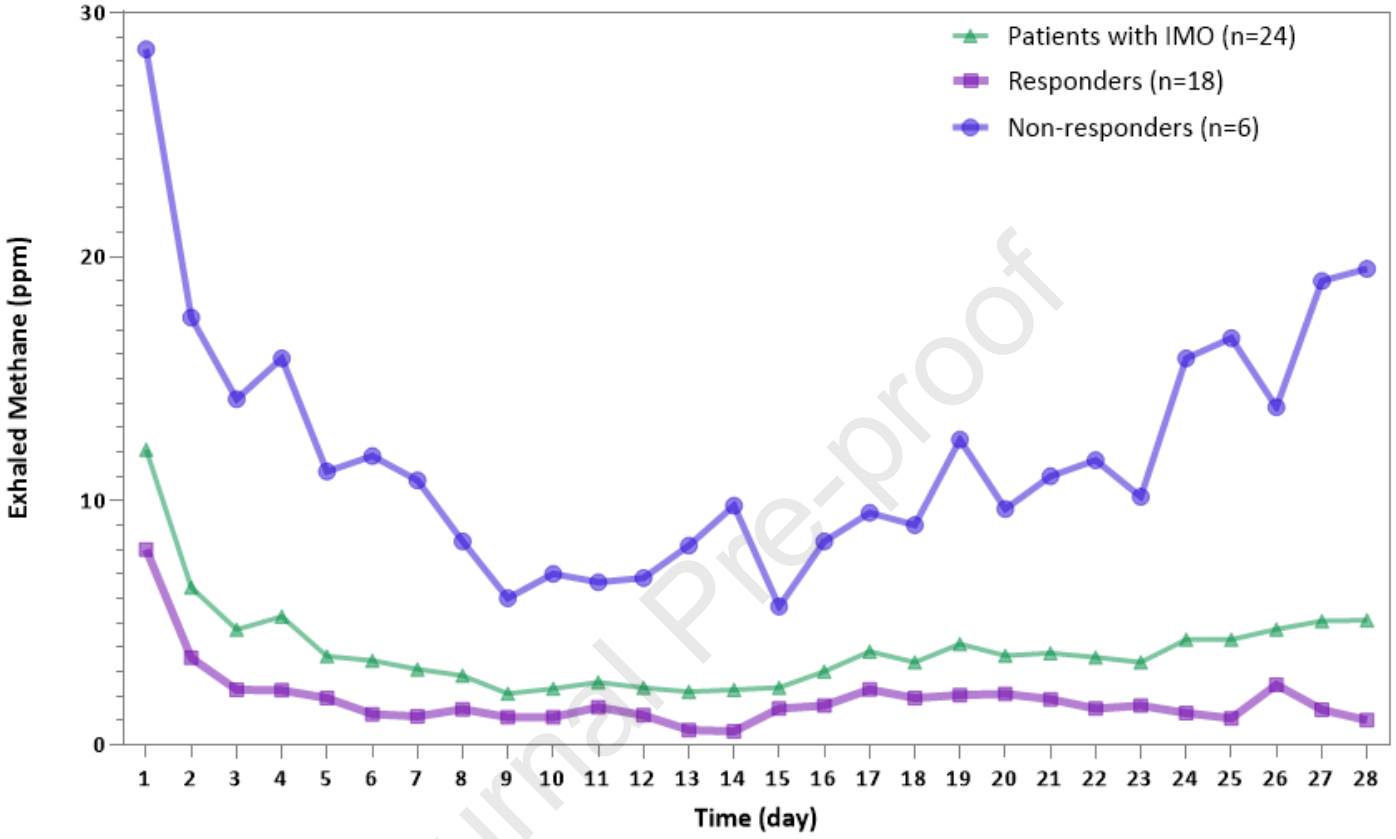


What You Need to Know

Background

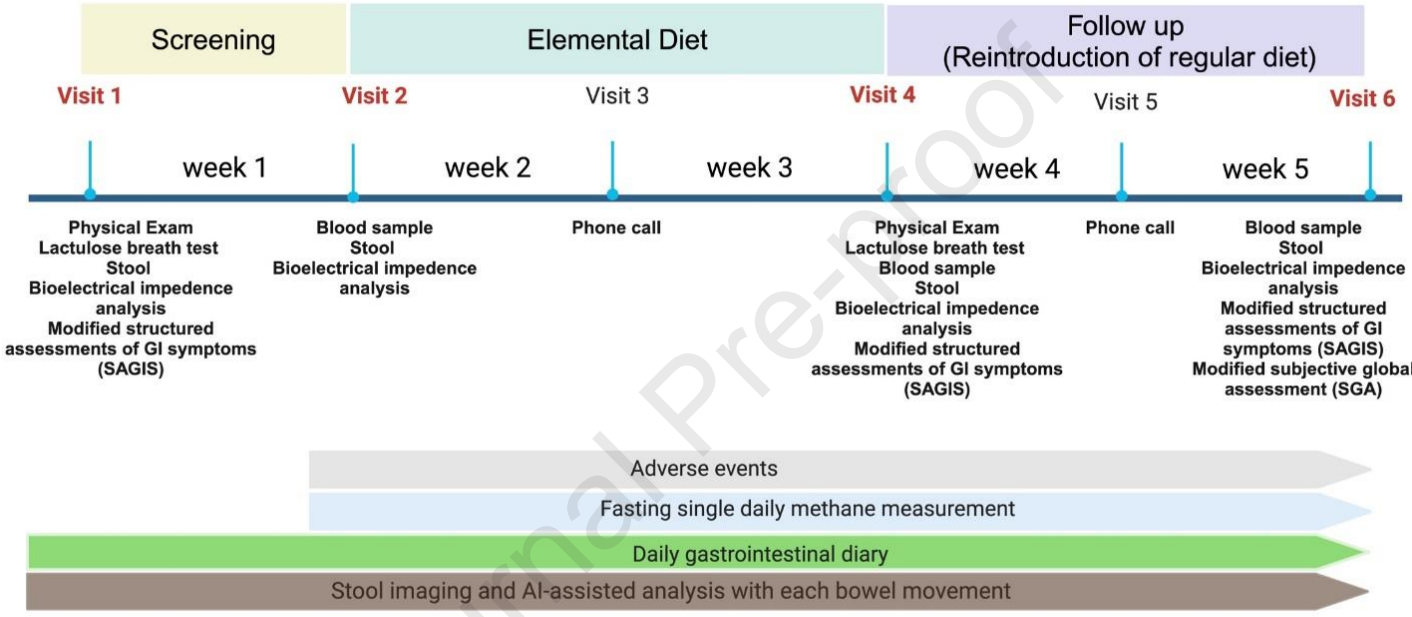
Elemental diets (EDs) have been explored to treat various gastrointestinal disorders including small intestinal bacterial overgrowth (SIBO) and intestinal methanogen overgrowth (IMO), but their poor palatability has limited clinical use.

Findings

A two-week exclusive PED course significantly modulated the gut microbiome, normalized breath tests in 73% of participants, and improved symptoms. No severe adverse events occurred, and 100% of participants tolerated the diet**.**

Implications for Patient Care

PED presents a well-tolerated, non-antibiotic therapeutic option for SIBO and IMO, offering an alternative or adjunct to standard treatments. Improved palatability may enhance patient adherence, broadening the clinical applicability of EDs.



NUTRITION INFORMATION
Serving Size 82 g / 6 Servings per Box (492 g)

	Amount Per Serving	Amount Per Box
Gram Weight	82 g	492 g
Calories	300 kcal	1800 kcal
Calories from Fat	18 kcal	110 kcal
Fat	2 g	12 g
Saturated Fat	2 g	12 g
Carbohydrates	62 g	370 g
Total Sugars	12.5 g	75 g
Sodium	215 mg	1300 mg
Potassium	565 mg	3400 mg
Protein	10 g*	60 g*
Vitamin A	150 mcg	900 mcg
Thiamin	0.2 mg	1.2 mg
Riboflavin	0.22 mg	1.3 mg
Niacin	2.7 mg	16 mg
Pantothenic Acid	0.83 mg	5 mg
Vitamin B6	0.3 mg	1.8 mg
Biotin	5 mcg	30 mcg
Folate	67 mcg	400 mcg
Vitamin B12	0.4 mcg	2.4 mcg
Vitamin C	15 mg	90 mg
Vitamin D	2.5 mcg	15 mcg
Vitamin E	2.5 mg	15 mg
Vitamin K	20 mcg	120 mcg
Calcium	200 mg	1200 mg
Chloride	334 mg	2005 mg
Chromium	5.8 mcg	35 mcg
Copper	0.15 mg	0.90 mg
Iodine	25 mcg	150 mcg
Iron	3 mg	18 mg
Magnesium	70 mg	420 mg
Manganese	0.40 mg	2.4 mg
Molybdenum	7.5 mcg	45 mcg
Phosphorus	116 mg	700 mg
Selenium	9.2 mcg	55 mcg
Zinc	1.8 mg	11 mg

* Contains free-form amino acids: 10 g/serving and 60 g/box

INGREDIENTS: Organic Maltodextrin, Dextrose, Medium Chain Triglycerides, L-Glutamine, Potassium Citrate, L-Threonine, L-Aspartic Acid, Natural Flavors, Glycine, Sodium Chloride, L-Leucine, Sodium Citrate, Dimagnesium Phosphate, L-Lysine, Calcium Carbonate, L-Tyrosine, L-Valine, L-Isoleucine, L-Methionine, L-Histidine, Taurine, L-Serine, Dicalcium Phosphate, L-Proline, L-Arginine, L-Cysteine, L-Tryptophan, L-Phenylalanine, Ascorbic Acid, Ferrous Sulfate, Niacinamide, Zinc Sulfate, dl-α-Tocopherol Acetate, Calcium D-Pantothenate, Manganese Sulfate, Pyridoxine Hy- drochloride, Riboflavin, Thiamine Hydrochloride, Copper Gluconate, Folic Acid, Vitamin A Palmitate, Biotin, Potassium Iodide, Ergocalciferol (Vitamin D2), Chromium Chloride, Sodium Ascorbate, Sodium Selenite, Menaquinone-7 (Vitamin K2), Sodium Molybdate, Cyanocobalamin (Vitamin B12).

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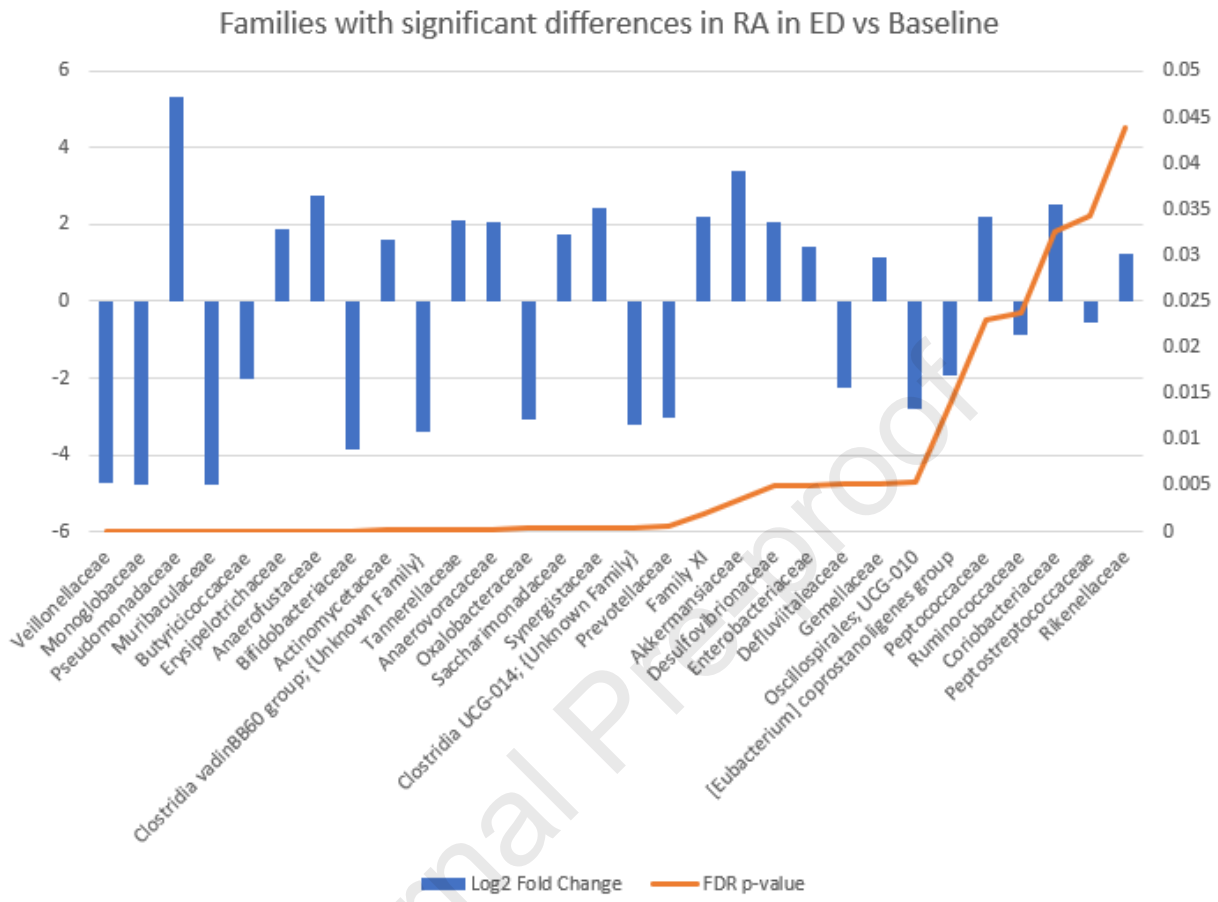

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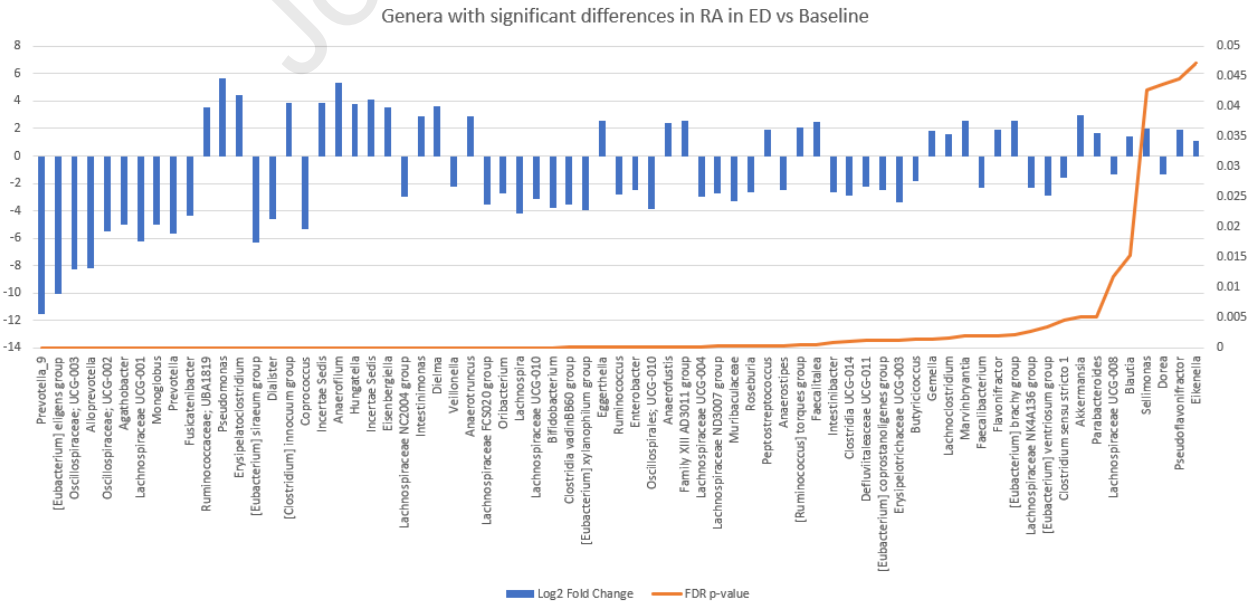

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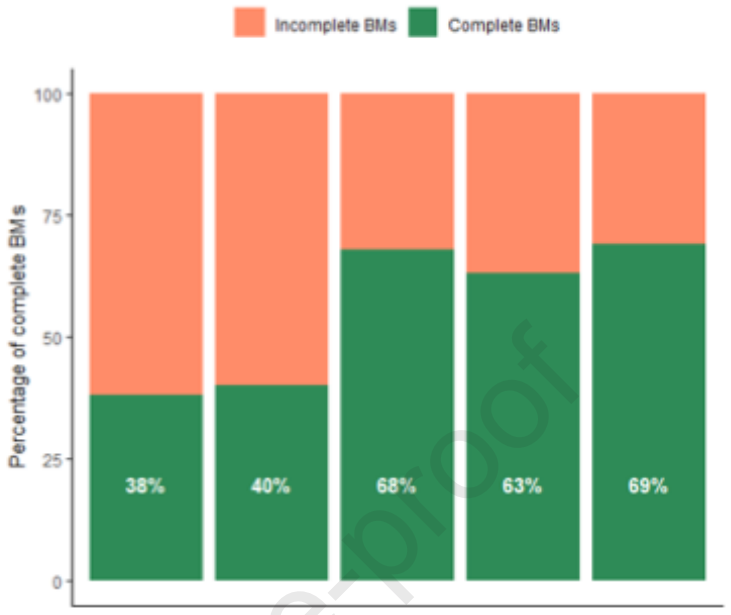

	Screening	PED wk 1	PED wk 2	F/U wk 1	F/U wk 2
	243	185	94	118	115
	147	124	202	200	252
Mean daily BMs (95% CI)	1.73 (1.33 – 2.13)	1.47 (1.00 – 1.94)	1.51 (1.15 – 1.87)	1.51 (1.15 – 1.87)	1.75 (1.35 – 2.15)

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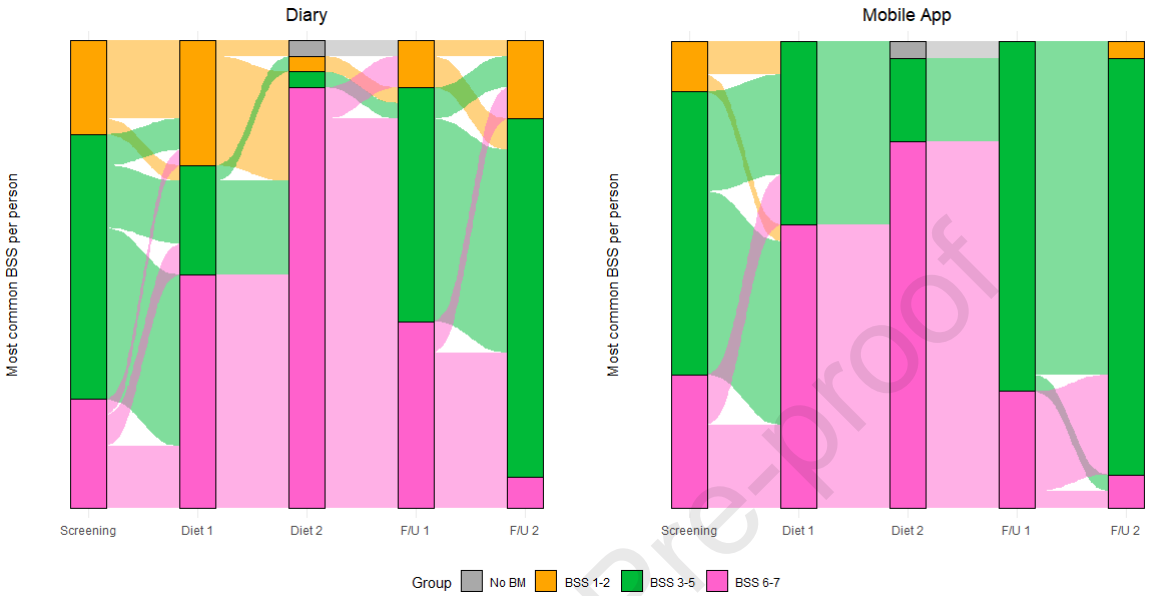

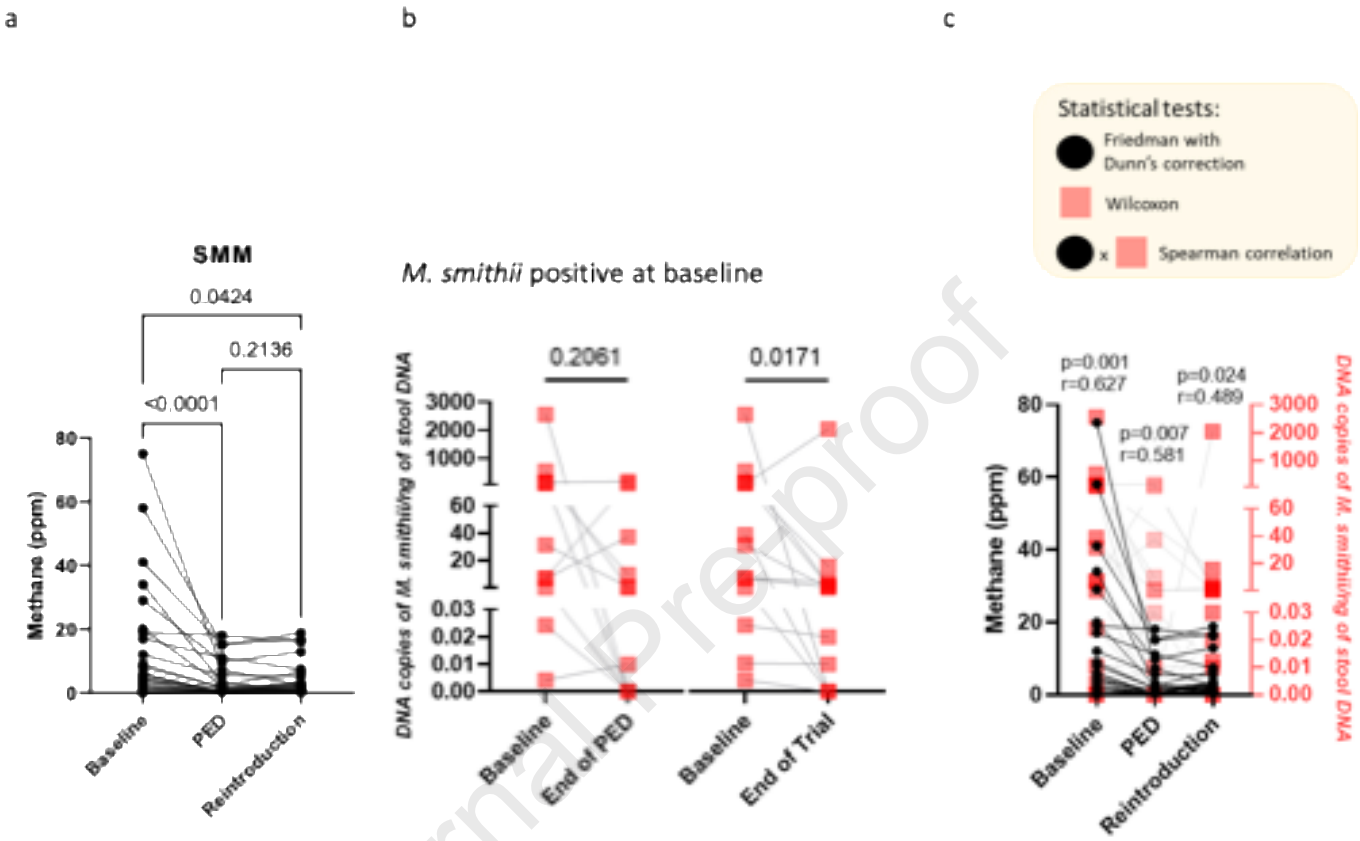

Supplemental Methods

Participants

Eligible subjects were symptomatic adults between 18 and 85 years of age with a positive 120-minute lactulose breath test (BT) for IMO, SIBO, or both (ClinicalTrials.gov ID: NCT05978973). Exclusion criteria included diabetes, pregnancy, breastfeeding, phenylketonuria, inflammatory bowel disease, eosinophilic GI disorder, active infection, or antibiotic use in the last month. Use of concomitant medications were allowed except for antibiotics. The protocol was approved by the Cedars-Sinai institutional review board and all subjects provided written informed consent. All authors had access to the study data and reviewed and approved the final manuscript.

Study design

Eligible subjects underwent one week of screening, two weeks of exclusive oral PED, and two weeks of follow-up after returning to their regular diet. (Supplemental Figure 1) The primary endpoint of the study was the change in the stool microbiome following PED and reintroduction of regular food. Secondary endpoints included organoleptic acceptability, tolerance, rate of normalization of BT, change in stool form, symptomatic response, and adverse events.

Intervention

The PED (mBiota Elemental, Good LFE, Santa Monica, USA) was provided in 300-calorie packets, tailored eucalorically to match each participant's daily caloric needs. (Supplemental Figure 2) The detailed composition of the diet is outlined in Supplemental Figure 2. Participants were given additional PED

packets and encouraged to consume more if they experienced hunger. Daily consumption of PED packets was documented by each participant. During PED treatment, there were no restrictions on water intake; however, participants were prohibited from consuming any other foods. Upon completing the diet phase, participants were advised to reintroduce bland, non-spicy foods, such as rice, potatoes, eggs, chicken, and beef, for 2 to 3 days before transitioning back to their regular diet.

Visits and data collection

Subjects were seen in person at baseline and weeks 1, 3 and 5. Telephone visits were performed at weeks 2 and 4 (Supplemental Figure 1). At baseline, subjects underwent a physical examination and completed a medical history questionnaire and modified structured assessment of gastrointestinal symptoms (SAGIS) questionnaire.[1] We modified British English spelling of SAGIS to American English. On a daily basis, subjects completed a gastrointestinal diary which recorded symptoms using a visual analog scale (VAS) on a scale of 0 (no symptom) to 100 (maximum symptom).[2] Additionally, subjects were asked to record their stool images for the entire duration of the trial using a validated HIPAA-compliant artificial intelligence-based smartphone application (Dieta AI stool tracker, Dieta health, USA) that objectively assesses stool characteristics using stool images.[3] Subjects also documented the time spent in the bathroom for each bowel movement.

After completion of the PED, subjects underwent a physical examination and completed modified SAGIS questionnaires[1] and symptom VAS. At the end of the trial, modified subjective global assessment (SGA)[4], and modified SAGIS questionnaires were completed.

Brain fog score was also calculated as the sum of five binary questions on confusion, cloudiness, impaired judgment, poor short-term memory, and difficulty with concentration.[5] Constipation score was calculated as the sum of three SAGIS 1-5 Likert scales for pain or discomfort prior to defecation, difficulty defecating (straining or incomplete evacuation of the bowel), constipation (reduced frequency of bowel movements, hard and lumpy stool). Adverse event queries and assessments were performed at weeks 1, 2, 3, 4, and 5.

Bioelectrical impedance analysis

Hands and feet were wiped with antibacterial tissue provided by the manufacturer prior to contact with electrodes in upright position. This position was held for the duration of the test where 30 impedance measurements with 6 frequencies (1, 5, 50, 250, 500, and 1000 kHz) were performed within 60 seconds. Total body weight, total body fat and visceral fat area were recorded.

Stool DNA isolation and quantification

Stool samples were collected at screening, after PED, and at the end of the trial were preserved in OMNIGENE Gut tubes (cat#OM-200, DNA Genotek, Sttitsville, Ontario, Canada) for DNA extraction using the MagAttract PowerSoil DNA EP Kit (#27100-4-EP, QIAGEN, Hilden, Germany), following the manufacturer's protocol. Briefly, 250 µl of stool samples were placed in the PowerBead DNA Plate and 750 µl of PowerBead/RNAse A solution to each well along with 60 µl of Solution SL and Garnet 0.7mm beads (#SKU 19-624, OMNI International, Kennesaw, Georgia, USA). The plate was sealed and homogenized in TissueLyzer II (#85300, QIAGEN, Hilden, Germany). The plate was centrifuged at 4500 x g for 6 minutes at room temperature. Supernatants were transferred to a Collection Plate and mixed with 450 µl of Solution IR. The plate was sealed, vortexed, incubated at 4ºC, and centrifuged. Supernatants were once again

transferred to a new Collection Plate and centrifuged; 450 µl of supernatants were transferred to a third Collection Plate, which was placed in the KingFisher Flex Purification System (#5400630, Thermo Fisher Scientific, Waltham, Massachusetts, USA) for DNA extraction. DNA was eluted in 100 µl of Solution EB and quantified by Qubit Qubit™ 4 Fluorometer (#Q33238, Thermo Fisher Scientific, Walthamm, Massachusetts), using the dsDNA BR Assay Kit (#Q32853, Thermo Fisher Scientific, Walthamm, Massachusetts, USA). 16S stool libraries were prepared according to the Illumina 16S Metagenomic Sequencing Library Preparation Protocol (Illumina, San Diego, CA, USA) (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf) using S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 primers described by Klindworth et al.[6] A total of 5 ng/µl of stool DNA in 2.5 µl was used to prepare each library. Final libraries were quantified using the Qubit 1x dsDNA HS Assay Kit (#Q33231, Thermo Fisher Scientific, Waltham, Massachusetts, USA) on Qubit™ 4 Fluorometer (#Q33238, Thermo Fisher Scientific, Walthamm, Massachusetts), and DNA fragment size was analyzed using the DNA 1000 Kit (#5067-1504), Agilent Technologies, Santa Clara, California, USA) on 2100 Bioanalyzer (#5065-4461, Agilent Technologies, Santa Clara, California). Each library was normalized to 4 nM using the 630bp fragment size, and samples were pooled into 2 separate multiplexed libraries containing half the samples, each. 6pM of pooled libraries and 15% PhiX were loaded on the Miseq platform (Illumina Inc., San Diego, California, USA) in each run.

At the end of the runs, FASTQ files were obtained and imported to CLC v.22.0.1 (QIAGEN, Hilden, Germany) for data analysis using the Microbial Genomics Module v.22.1.2 as described previously [7].

Taxonomic terms were assigned by comparing sequences to the SILVA database v.138.1, with a similarity percentage of 97%; terms with a combined abundance of less than 3 were filtered out. OTUs classified as " gut metagenome", "metagenome", "human", "cyanobacteria", "mitochondria", "uncultured bacterium", "unidentified organism", and "ambiguous taxa" were filtered out to prevent taxa misidentification. Differences in relative abundance (RA) were compared at the family and genus level by performing the Generalized Linear Model on groups and using the Wald test to determine significance; rare genera (RA median < 1 in both groups) were removed.

OTU tables were obtained, and microbiome evenness and richness were compared using the Shannon diversity index as a measure of alpha diversity. According to normalization strategies described in Weiss et al. [13] rarefaction was not used as no difference in library size was observed between the groups (data not shown).

Bray-Curtis dissimilarity (β-diversity) and alpha-diversity t-test, analysis of data distribution, and correlations were performed on GraphPad Prism v9.5.1 (GraphPad Software, Boston, Massachusetts, USA). Shapiro-Wilk normality test was used to analyze data distribution. Pearson correlation was performed when both variables showed a normal distribution; otherwise, Spearman correlation was applied. Alpha diversity measurements were obtained from CLC Genomics Workbench v.22 and compared between groups by t-test in GraphPad Prism v.9.5.1.

Methanobrevibacter smithii (M. smithii) is the most abundant methanogen in humans.[8] The presence of *M. smithii* in stool samples was examined by dPCR in a QIAcuity Four Platform System (QIAGEN, Hilden, Germany) using custom specific Taqman primers and probes (20X, Thermo Fisher Scientific, Walthamm,

Massachusetts, USA) and the QIAcuity Probe Master Mix (#250102, 4X, QIAGEN, Hilden, Germany) in a QIAcuity Nanoplate 26k 24-well (#250001, Qiagen, Hilden, Germany). The primer and probe sequences for *M. smithii* rpoB gene (product size = 70bp) were obtained from Dridi et al. [9]: Ms_rpoBF, 5'-AAGGGATTTGCACCCAACAC-3'; Ms_rpoBR, 5'-GACCACAGTTAGGACCCTCTGG-3'; Ms_rpoBFAM, 5'-ATTTGGTAAGATTTGTCCGAATG. Each well contained 40 µL of a mix of 2.2 µL of Taqman primers and probe mix, 11 µL of QIAcuity Probe Master Mix, 24.6 µL of ultrapure water, and 4.2 µL of DNA (samples or positive control). The dPCR conditions were 95ºC for 2 minutes, 40 cycles at 95ºC for 30 seconds and 60ºC for 60 seconds, and an additional cooling down step at 40ºC for 5 minutes. The imaging parameters were 500 milliseconds of exposure, and 6 of gain in the green channel. Results were reported in copies of *M. smithii* per ng of stool DNA. A total of 29 and 27 DNA samples from stool were analyzed at baseline and end of the trial, respectively. Wilcoxon test was performed to compare *M. smithii* levels between baseline and end of the trial (Follow up) groups.

BT and fasting single daily methane measurements (SMM)

Preparation for LBT included avoidance of highly fermentable products 24-hours before the test and fasting overnight. Breath samples were collected via a dual bag collecting system (Quintron Instrument Company, Milwaukee, WI). Sampling was performed at baseline and every 15 minutes for up to 120 minutes after the ingestion of 10g lactulose followed by ~120 mL of water. The sampled gas was analyzed using a BreathTracker SC (Quintron Instrument Company, Milwaukee, WI). Output was reported as hydrogen (H_2) and methane (CH_4) in parts per million (ppm) after correction for alveolar sample quality using breath CO_2 concentration. Abnormal LBTs were classified as SIBO (H_2 rise of ≥20 ppm within 90 minutes); IMO (CH_4

of ≥10 ppm at any timepoint); IMO/SIBO (meeting both SIBO and IMO criteria described above), flatline test (nonmethane fixed-hydrogen producers), or elevated baseline ($H_2 \geq 20$ ppm at baseline).[10-12] LBT was considered normal if none of the aforementioned criteria were met.[10] Upon consumption of the PED to the end of the study, subjects collected single fasting breath samples at home every morning by blowing into a specialized test tube (Extainer, Labco, Ceredigion, UK) for determination of fasting single daily methane measurements (SMM). The response in patients with IMO, as determined by SMM, was defined as achieving a level ≤5 on the final day of the trial.[13]

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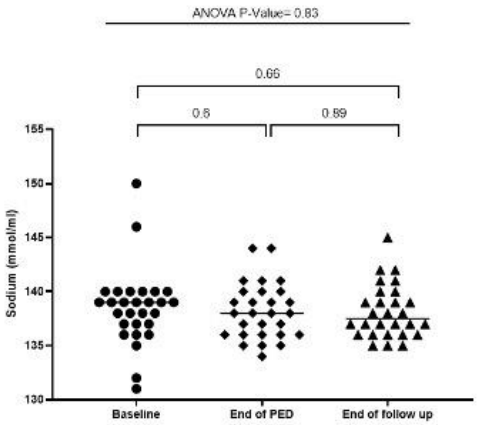

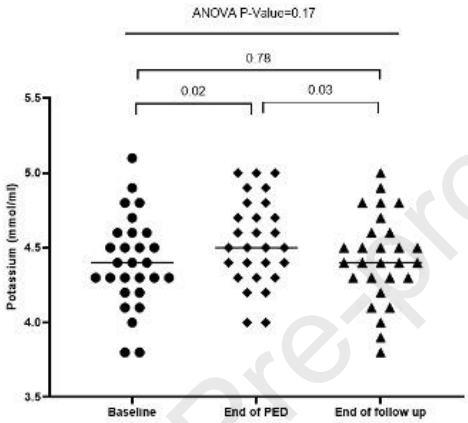

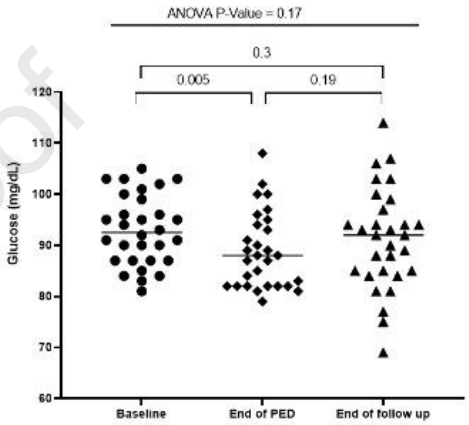

Supplementary figure 1. Study design and time points.

Supplementary figure 2. Composition of the palatable elemental diet.

Supplementary figure 3. a) Bacterial families with significant changes in relative abundance after 2 weeks of elemental diet as compared to baseline. b) Bacterial genera with significant changes in relative abundance after 2 weeks of elemental diet as compared to baseline.

Supplementary figure 4. a) Weekly number and percentage of complete bowel movements for each 5 weeks of the trial. Significant improvement in completeness was observered from the second week of the palatable elemental diet (PED). b) Weekly changes in the most common Bristol Stool Scale (BSS) based on daily diaries and objectively determined by artificial intelligence from the stool images.

Supplementary figure 5. Single fasting daily methane measurements (SMM) in the breath and copies of *M. smithii* in the stool of subjects with IMO or IMO/SIBO (n=24). a) Friedman paired test with Dunn's correction was used to compare SMM levels from baseline to the average of SMMs recorded during the PED phase and the average of SMMs recorded during the reintroduction of regular food; b) Copies of *M. smithii*/ng of stool DNA at baseline and the end of the PED (n=11) and reintroduction phases (n=13) in subjects who were positive for *M. smithii* at baseline. DNA copies of *M. smithii*/ng of stool DNA were compared using Wilcoxon paired test; c) Spearman correlation of DNA copies of *M. smithii*/ng of stool DNA with SMMs at baseline, PED and reintroduction phases.

Supplementary figure 6: Sodium, potassium, and glucose levels measured during visits 2 (screening), 4 (upon 2 week of palatable elemental diet), and 6 (2 weeks after reintroduction of regular diet). Electrolytes remained within normal limits at visits 4 and 6 and no episodes of hypo- or hyperglycemia were observed.