**Title:** Histological improvement of non-alcoholic steatohepatitis with a prebiotic: a pilot clinical trial.

**Authors:** Marc R. Bomhof 1,2, Jill A. Parnell3, Hena R. Ramay4, Pam Crotty5, Kevin P. Rioux5,6, Chris S. Probert7, Saumya Jayakumar5, Maitreyi Raman5, and Raylene A. Reimer2,8

**Author affiliations**

1Department of Kinesiology and Physical Education, 4401 University Drive, University of Lethbridge, Lethbridge, AB, Canada, T1K 3M4

2Faculty of Kinesiology, 2500 University Drive NW, University of Calgary, Calgary, AB, Canada T2N 1N4

3Department of Health and Physical Education, 4825 Mount Royal Gate SW, Mount Royal University, Calgary, AB, Canada T3E 6K6

4International Microbiome Centre, Cumming School of Medicine, Health Sciences Centre, 3280 Hospital Drive NW, University of Calgary, Calgary, AB, Canada T2N 4Z6

5Division of Gastroenterology and Hepatology, Department of Medicine, 3280 Hospital Drive NW, University of Calgary, Calgary, AB, Canada T2N 4Z6

6 Immunology and Infectious Diseases, Department of Microbiology, 1863 Health Sciences Centre, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4Z6

7Institute of Translational Medicine, Crown Street, University of Liverpool, Liverpool, United Kingdom, L69 3BX

8Department of Biochemistry and Molecular Biology, University of Calgary, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1

**Corresponding author:** Dr. Raylene Reimer, PhD, RD. Faculty of Kinesiology, University of Calgary, 2500 University Drive NW. Calgary, AB, Canada T2N 1N4. Email: reimer@ucalgary.ca. Phone: (403) 220-8218. Fax: (403) 220-2070

**Author contributions:**MRB executed study, collected data, analyzed data, and prepared the manuscript; JAP designed study and obtained funding; HRR analyzed 16S gut microbiota sequencing data; PC collected data; KPR, SJ, and MR designed study, collected data, and recruited participants; CSP performed VOC analysis; RAR designed study, obtained funding and had final responsibility for the study. All authors had access to the study data and reviewed and approved the final manuscript.

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**Abstract**

*Purpose:* In obesity and diabetes the liver is highly susceptible to abnormal uptake and storage of fat. In certain individuals hepatic steatosis predisposes to the development of non-alcoholic steatohepatitis (NASH), a disease marked by hepatic inflammation and fibrosis. Although the precise pathophysiology of NASH is unknown, it is believed that the gut microbiota-liver axis influences the development of this disease. With few treatment strategies available for NASH, exploration of gut microbiota-targeted interventions is warranted. We investigated the therapeutic potential of a prebiotic supplement to improve histological parameters of NASH.

*Methods:* In a placebo controlled, randomized pilot trial, 14 individuals with liver biopsy confirmed NASH (Non-alcoholic fatty liver activity score (NAS) ≥ 5) were randomized to receive oligofructose (8 g/day for 12 weeks followed by 16g/day for 24 weeks) or isocaloric placebo for 9 months. The primary outcome measure was the change in liver biopsy NAS score and the secondary outcomes included changes in body weight, body composition, glucose tolerance, inflammatory markers, and gut microbiota.

*Results:* Independent of weight loss, oligofructose improved liver steatosis relative to placebo and improved overall NAS score (P=0.016). *Bifidobacterium* was enhanced by oligofructose whereas bacteria within *Clostridium* cluster XI and I were reduced with oligofructose (P<0.05). There were no adverse side-effects that deterred individuals from consuming oligofructose for treatment of this disease.

*Conclusions*: Independent of other lifestyle changes, prebiotic supplementation reduced histologically-confirmed steatosis in patients with NASH. Larger follow-up studies are warranted.

*Clinical Trial*: This trial was registered at Clinicaltrials.com as NCT03184376.

**Keywords:** non-alcoholic steatohepatitis, gut microbiota, prebiotic, oligofructose

**Introduction**

Non-alcoholic fatty liver disease (NAFLD) has increased in parallel with the rise in obesity [1]. It is estimated that 75% of adults and 34% of children with obesity are affected by NAFLD [2,3]. The liver is susceptible to increased storage of fat in obesity in part due to metabolic complications associated with insulin resistance in peripheral tissues [1]. An inflammatory cascade is triggered in 10-25% of individuals with NAFLD leading to non-alcoholic steatohepatitis (NASH), an advanced form of NAFLD that jeopardizes liver function and can lead to cirrhosis, hepatocellular carcinoma, and end stage liver failure [1]. Given evidence that a 10% reduction in weight improves histological parameters of this disease, weight loss through diet and exercise is generally prescribed [4]. However, with low success rates for sustained weight loss with lifestyle management [5], alternative treatment strategies are warranted.

Although the pathophysiology of NASH is not completely understood, it has been proposed that in NAFLD the liver is vulnerable to parallel ‘hits’ stemming from localized oxidative stress and pro-inflammatory agents from peripheral tissues [6]. Gut microbiota dysbiosis is hypothesized to be one of the major ‘hits’ contributing to NASH [7,8]. By compromising intestinal permeability, gut microbiota dysbiosis permits an increased flux of lipopolysaccharide (LPS), a potent inflammatory agent, into portal circulation [8]. The gut microbiota also exerts a significant influence on host energy extraction, appetite, and adiposity [9]. Based on evidence demonstrating that a dysbiotic gut microbiota can induce NAFLD in germ free mice, it has even been suggested that gut microbiota may play a causal role in the pathogenesis of this disease [10]. With new and emerging research highlighting the altered gut microbiota profiles in individuals with NAFLD [11] there is considerable interest in exploring gut microbiota-based strategies to halt and/or reverse the progression of NASH.

Prebiotics are substrates that are ‘selectively utilized by the host microorganisms conferring a health benefit’ [12]. Inulin-type fructans, a class of prebiotic, are found naturally in foods including asparagus, garlic, leeks, artichokes, and onions and are increasingly being incorporated into functional foods [13]. By increasing the growth and activity of health promoting bacteria, prebiotics offer a safe and effective means of altering the gut microbiota [12]. Animal and human research has demonstrated that the prebiotic oligofructose improves several NAFLD-associated metabolic risk factors including gut microbiota dysbiosis, intestinal permeability, endotoxemia, inflammation, glycemia, and hepatic lipogenesis [14-18]. Studies, however, have not thoroughly investigated the clinical potential of oligofructose to affect histological measures of steatosis, lobular inflammation, and hepatocellular ballooning, the current gold standard for diagnosing NASH [1]. The objective of this pilot study was to investigate the effectiveness of the prebiotic oligofructose for improving histological features in liver biopsy-confirmed NASH (NAS ≥5) [19]. Secondary outcomes included changes in body composition, glucose tolerance, serum lipids, inflammatory markers, and gut microbiota.

**Participants and methods**

**Participants**

Participants with liver biopsy confirmed NASH (NAS score ≥5) were identified and recruited at the University of Calgary Foothills Medical Centre (Calgary, AB, Canada). Additional inclusion criteria were: males and females ≥18 years old, body mass index (BMI) >25 kg/m2 (Caucasians) and >23 kg/m2 (Asians), history of Serum ALT >1.5X upper normal limit, no changes in lipid-lowering or diabetes medication over previous three months, and ability to provide informed consent. Exclusion criteria were: alcohol consumption >20g/day (women) or >30g/day (men), alternate etiology for abnormal liver enzymes, decompensated liver disease, and use of orlistat, liraglutide, prebiotic, probiotic, or antibiotic within 3 months prior to enrollment. Eligibility was assessed using a screening questionnaire and interview conducted by a research coordinator. If a subject met eligibility criteria, written and informed consent was obtained. A flow diagram of subject recruitment, randomization, and analysis is provided in Fig. 1. Ethical approval for the study was obtained from the Conjoint Health Research Ethics Board of the University of Calgary (Ethics ID #E-23936) and the study performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This trial was registered at Clinicaltrials.com as NCT03184376.

**Study design and diet intervention**

Following screening, participants were randomly assigned to one of two groups by a senior study investigator not involved in recruiting participants. Participants were randomized into either a treatment group that received oligofructose (Orafti P95, Beneo-Orafti Inc., Tienen, Belgium) prebiotic (PRE) 8g orally per day for 12 weeks followed by 16g per day for 24 weeks or an isocaloric maltodextrin placebo (PLA) control. Given evidence that some individuals experience increased flatulence and gastrointestinal discomfort as a result of oligofructose intake [20], the initial 8gram dose was used to promote gastrointestinal adaptation and help participants adjust to the oligofructose supplementation. Oligofructose and maltodextrin have a similar taste and look and both were provided to participants in identical packets. Participants were blinded to the treatment allocation. Adherence to supplement protocol was monitored throughout the study through direct questioning and counting of unused supplement packets. Participants were asked to maintain their usual physical activity.

Body weight, waist circumference, body composition via dual energy x-ray absorptiometry (DXA) scan (QDR 4500, Hologic, Inc. Bedford, MA, USA), dietary intake, fasting blood chemistries, glycemia, and gut microbiota were serially measured at baseline, week 12, week 24 (excluding DXA), and week 36. Fasting blood work and oral glucose tolerance tests (OGTT) consisting of a 75g glucose beverage and follow-up blood collections at 60min and 120min were conducted at one of the Calgary Laboratory Services clinics located throughout Calgary, AB, Canada.

Food intake was assessed using 3 day food records (one weekend day and two weekdays) recorded by participants every 12 weeks. Food records were analyzed using FoodWorks 14 Software (The Nutritional Company, Long Valley, NJ, USA).

A modified Godin’s leisure time exercise questionnaire (GLTEQ) was used to determine the number of hours of mild, moderate, and strenuous activity completed by each participant [21]. Metabolic Equivalent (MET)•hours was determined for each subject by multiplying the number of hours of mild, moderate, and strenuous activity by an estimated MET value for each level of activity (Mild: e.g. easy walking - 3 MET•hours; Moderate: e.g. brisk walking – 5 MET•hours; Strenuous: e.g. running – 9MET•hours).

Post-study subjective evaluation of acceptability, convenience, tolerability, and hunger was determined using 100mm visual analog scales (VAS). Scores for each question were compared between groups.

Prior to enrolling in the study (initial) and upon study completion (final) all participants underwent an ultrasound guided liver biopsy at the University of Calgary Foothills Medical Centre. The initial liver biopsy was performed as standard of care by clinicians at the University of Calgary Foothills Medical Centre to diagnose and classify NAFLD. Only patients with confirmation of NASH (NAS ≥ 5) were approached by clinicians regarding participation in this study. The GI pathologist at the University of Calgary Foothills Medical Centre (blinded to treatment allocation) assigned all biopsies a NAS score based on a standardized grading system for steatosis (scale of 0-3), lobular inflammation (scale of 0-3), and hepatocellular ballooning (scale of 0-2). Higher scores indicate increasing severity of disease, with NAS score of ≥ 5 indicating NASH [19].

**Blood biochemistries**

Fasting concentrations of alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP) and serial measurements of glucose from the OGTT were measured by Calgary Laboratory Services (Calgary, AB). Fasting concentrations of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, both secreted from adipocytes and inflammatory cells and purported to play a role in NASH pathogenesis through recruitment of macrophages to the liver and activation of both Kupffer and hepatic stellate cells [22], as well as serial measures of insulin from serum collected during the OGTTs were measured in duplicate using the Human Adipokine Milliplex Panel-B kit (HADK2MAG-61K, Millipore, St. Charles, MO, USA). Eve Technologies (Calgary, AB, Canada) completed plate analysis using Luminex 200 instrumentation and software (intra-assay %CV <10% and inter-assay %CV <15% for all analytes). Insulin resistance was approximated using the homeostasis model assessment of insulin resistance (HOMA2-IR) [23] and the Matsuda index [24]. LPS was measured using a PyroGene Recombinant Factor-C assay (Lonza, Walkersville, MD, USA) with serum samples diluted 1/50 and heated for 15 minutes (70°C) prior to analysis.

**Stool collection and gut microbiota analysis**

Stool was collected using a home-use kit (Protocult, Rochester, MN, USA). Samples were placed in a biohazard container with icepacks and immediately placed in a -20°C freezer. Within 48h, samples were delivered to the lab and placed into a -80°C freezer. Gut microbiota was analyzed using 16S rRNA Illumina sequencing and qPCR [25,26]. A detailed description of the methods is provided in the ESM\_ Supplementary Methods.

**Volatile organic compound (VOC) analysis**

VOC analysis was conducted at the Institute of Translational Medicine at the University of Liverpool (Liverpool, UK). Protocol details are provided in ESM\_ Supplementary Methods.

**Statistical analysis**

Data is expressed as mean ± SEM. For outcome variables measured at baseline and week 36, mean difference (post minus pre-treatment) values were used to compare groups using an independent t-test or Mann-Whitney U test. Within group differences were determined using a paired t-test or Wilcoxon signed-rank test. Data with serial measures at week 0, 12, 24, and 36 was analyzed using mixed design (split-plot) one-way repeated measures ANOVA. Data that was not normally distributed was log10 transformed. Correlations were assessed using Spearman’s correlation test. Analysis was completed on an intent-to-treat basis. Data analysis was performed using SPSS 21.0 (IBM, Armonk, NY, USA). Phyloseq R package was used for bacterial sequencing analysis [27]. Alpha diversity was measured by calculating the Shannon and Simpson indices. Beta diversity was evaluated using non- metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity matrix. The OTU table was transformed using the variance stabilization transformation available in the DESEq2 package [28] for repeated measures ANOVA. VOC analysis was performed using R version 3.1.2 or the online package Metaboanalyst <http://www.metaboanalyst.ca/faces/ModuleView.xhtml>. A false discovery rate correction was applied to bacterial and VOC analysis to control for type I error. P<0.05 was considered statistically significant.

**Results**

**Subject characteristics**

A total of 14 individuals were recruited and of these, 14 completed the study (Fig. 1). PRE was composed of 8 participants (3 female, 5 male) with an average age of 45.3y ± 5.6. PLA was composed of 6 participants (3 female, 3 male) with an average age of 53.3y ± 4.8. Participant baseline characteristics and demographics are detailed in Table 1 and ESM\_Table S1. Baseline age, body weight, BMI, and body composition did not differ between PRE and PLA.

**Anthropometrics**

There were no differences in repeated measures of body weight, BMI, waist circumference, or body composition between PRE and PLA across the 36 week intervention (Table 1).

**Prebiotic improves histological parameters of NASH**

NAS and fibrosis scores obtained from the initial liver biopsies completed prior to study enrollment were not different between PRE (Steatosis: 2.88 ± 0.13; Inflammation: 1.63 ± 0.18; Ballooning: 1.38 ± 0.18; Total NAS: 5.88 ± 0.30; Fibrosis: 1.38 ± 0.32) and PLA (Steatosis: 2.80 ± 0.20; Inflammation: 1.40 ± 0.24; Ballooning: 1.20 ± 0.20; Total NAS: 5.40 ± 0.24; Fibrosis: 2.20 + 0.37). All 14 participants completed a 36 week, post intervention follow-up liver biopsy. One biopsy from the PLA group was excluded at week 36 due to the pathologists’ indication of an inadequate sample for histology. PRE had a significant within-group and between-group decrease in hepatic steatosis (Fig. 2). Lobular inflammation was reduced with PRE, however the decrease did not reach statistical significance (P=0.059). There was a significant decrease within PRE for the total NAS (P=0.016). Fibrosis scores were not affected by PRE and PLA.

**Blood markers of liver function, inflammation, and glycemic control**

PRE and PLA did not affect serum ALT, GGT, or ALP concentrations (Table 2). There was, however, a positive correlation between delta NAS and delta ALT (r=0.611, P=0.027). Although there was a 42% and 15% decrease in IL-6 and TNF-α respectively with PRE, this was not significantly different due to our low sample size. LPS was numerically lower in PRE and higher in PLA at the end of the 36 week trial but this did not reach significance (Table 2). No improvement in glycemic control was observed during the trial. The lower fasting glucose (Table 2) and lower glucose concentrations over the course of 4 OGTTs (ESM\_Fig. S1A) in PRE relative to PLA was largely driven by initial baseline differences. Serum insulin levels and insulin sensitivity according to the HOMA2-IR and the Matsuda index remained the same between PRE and PLA during the study (Table 2). Insulin levels during the OGTTs, although not statistically different, tended to be lower in PLA (ESM\_Fig. S1B).

**Characterization of gut microbiota and VOCs**

*qPCR analysis*

Over 36 weeks, PRE increased *Bifidobacterium* spp. abundance (week × treatment P=0.017) (Fig. 3C) and reduced *Clostridium* cluster XI (week × treatment P=0.030) relative to PLA (Fig. 3D; ESM\_Table S2). Although *C.* cluster I was higher in PRE compared to PLA at the start of the study, PRE reduced *C.* cluster I, leading to similar abundance of *C.* cluster I at the end of the 36 week trial (week × treatment P=0.032) (Fig. 3E). PRE and PLA were both associated with an increase in *C. leptum* and *Faecalibacterium prausnitzii* over the course of the study (week P=0.017).

*16S rRNA sequencing*

With PRE there was an increase in the relative abundance of Actinobacteria (week × treatment P=0.045) (Fig. 3A) and Bifidobacteriaceae (week × treatment P=0.037) (ESM\_Fig. S2A,B); however, these increases were not statistically significant after applying a False Discovery Rate correction. No differences in beta-diversity (Fig. 3B) or alpha diversity (ESM\_Fig. S2C,D) were observed between PRE and PLA.

*VOC analysis*

Overall, there was no separation or significant differences in fecal VOCs between PRE and PLA (ESM\_Fig. S3A). The three VOCs showing the greatest difference between PRE and PLA were p-Xylene, 1-Butanol, and oxalic acid butyl propyl ester (ESM Fig. S3B,C,D)

**Food intake and physical activity**

Energy and dietary fiber (excluding treatment supplements) intake were not different between PRE and PLA (ESM\_ Table S3). MET•hours was lower in PRE relative to PLA at baseline (ESM\_Table S3). At study completion MET•hours were equivalent between groups. The lack of an interaction between week and treatment suggests that changes in activity were not differentially affected over the course of the study in PRE and PLA.

**Acceptability of treatment**

There were no differences in VAS evaluation of treatment acceptability, convenience, tolerability, and hunger between PRE and PLA (Table 3). PRE and PLA participants agreed that supplement packages were convenient and acceptable for long-term use. One participant in both PRE and PLA cited increased flatulence as a deterrent for supplement consumption. Overall, there was a trend towards individuals in PRE having a higher degree of fullness throughout the day relative to PLA (P=0.059).

**Discussion**

We investigated the therapeutic potential of oligofructose to improve histological features of NASH in a randomized, placebo controlled pilot clinical trial. Despite no changes in body weight or composition, oligofructose supplementation resulted in a significant decrease in steatosis and overall NAS. Corresponding with these changes, oligofructose increased *Bifidobacterium* and decreased *C*. cluster I and XI. While sample size was limited in this pilot study, this evidence supports oligofructose as a well-tolerated strategy to modify gut microbiota targeted at management of NASH.

Mounting evidence for the role of gut microbiota in energy regulation and inflammation has triggered interest in evaluating how manipulation of the microbiota might help manage chronic diseases, including NASH. An early study by Daubioul et al. assessed the effects of oligofructose (16g/day) for 8 weeks in a randomized, double-blind, cross-over study in 7 individuals with liver biopsy confirmed NASH [17]. OFS was demonstrated to significantly reduce aspartate aminotransferase (AST), however follow-up histological measures were not assessed. In one of the few gut-microbiota based intervention studies to include a follow-up liver biopsy, a placebo-controlled trial by Malaguarnera et al. assessed the effects of a synbiotic containing *Bifidobacterium* *longum* (dose unspecified) with 2.5 g fructo-oligosaccharides (FOS) in combination with lifestyle management in NASH [29]. After 24 weeks, *B. longum* and 2.5g FOS treatment improved serum biomarkers and reduced liver steatosis, hepatocellular injury, inflammation, fibrosis, and overall NASH activity. Additional studies using both synbiotics [30] as well as probiotics [31-33] have demonstrated improvement in serum biomarkers in NASH. Here, using a liver biopsy to assess the histological markers of NASH after 9 months, we observed a reduction in steatosis relative to placebo and an overall within-group reduction in NAS, although we did not observe any changes in serum markers. The study by Malaguarnera et al. did not examine gut microbiota and thus it is not possible to ascertain the extent to which the gut microbiota was altered and/or played a role in the improvements seen with the synbiotic. The 2.5g dose of FOS used by Malaguarnera et al. is a relatively low dose of prebiotic compared to the 16g dose used in our study. Furthermore, although the dose of *B. longum* was not specified, other research with a synbiotic in an animal model showed that oligofructose elicited greater modifications to gut microbiota than the probiotic alone [34]. While overall NAS was reduced in our study, a reduction in fibrosis was not observed which is consistent with findings from other intensive weight loss and lifestyle interventions [35].

Despite altered hepatic histopathology in NASH with oligofructose, body weight and body composition did not change over the 9 month intervention in this population. While our research group as well as others have demonstrated modest weight loss with prebiotics in otherwise healthy adults [36,37] and children [26] with overweight and obesity, this finding is not unanimous. A study by Daud et al. assessed the effects of 30g oligofructose versus cellulose for 8 weeks in individuals with overweight and obesity [38]. Similar to our current observations, no changes in body weight or body composition were observed. Interestingly, in keeping with our results, the authors indicated that there was a trend towards reduced intrahepatocellular lipids measured with magnetic resonance imaging, although their participants did not have NAFLD. In another study, Dewulf et al., found that a 16g/day mixture of inulin/oligofructose did not affect body weight in women with obesity, although there was a trend toward a decrease in fat mass with oligofructose [16]. Given the histological improvement observed with oligofructose, it would appear that many of the benefits are weight independent. While it has been observed that weight loss and improvement of NASH are correlated [35], weight loss is not always necessary to facilitate an improvement in NASH [39].

Numerous studies have demonstrated that NASH is characterized by an altered gut microbiota [11]. Using 16S rRNA pyrosequencing, Zhu et al. demonstrated that NASH in a pediatric population is characterized by decreased Actinobacteria and *Bifidobacterium* [40]. In contrast, in an adult population, differences in *Bifidobacterium* were not observed [41]. Here we showed that oligofructose increased *Bifidobacterium* in NASH. Through increases in *Bifidobacterium*, a bacteria identified to be inversely associated with obesity [42] and plasma LPS [16], oligofructose may target multiple metabolic pathways that converge on an improvement in NASH (see reference [15] for a review). Although pathogenic species can arise from *C*. cluster I and cluster XI, the potential benefits of reductions in these clusters in NASH that was seen with oligofructose remains unclear.

The success of any dietary intervention requires long-term adherence. Individuals in both PRE and PLA indicated that the supplements were convenient and acceptable for long term use. One participant in PRE and PLA reported increased flatulence with supplement use. The explanation for increased flatulence with maltodextrin, a non-fermentable carbohydrate that is fully absorbed in the small intestine, is not clear but has been reported previously[16]. It may be possible that participants, upon initiation of a study protocol, pay closer attention to bowel habits and thus perceive increased gastrointestinal symptoms. The dose of 16g/day oligofructose is in general accordance with limits of tolerance. While there are inter-individual differences, research suggests that in a population with a BMI <25 kg/m2 the dose after which gastrointestinal symptoms develop with fructo-oligosaccharide is between 10-20g [20]. It is not clear, however, whether individuals with obesity have an enhanced or reduced gastrointestinal response to fructo-oligosaccharide consumption. Daud et al. in their clinical trial provided a 30g/day dose of oligofructose [38]. While this dose was tolerated by the majority of participants, 4 participants had to temporarily decrease their dose to 20g/day. Based on our study and evidence from Dewulf et al. [16], 16g/day is tolerable and appropriate in NASH.

This study was limited by several factors. Although liver biopsy is the gold standard for diagnosing NASH, liver biopsies can be limited by sampling error [43]. Furthermore, the invasive nature of liver biopsies makes it a challenge to recruit participants. With a low sample size we had limited statistical power to discern differences between groups and we could not anticipate that subjects with poorer glycemic control would cluster in the PLA arm and gut microbiota profiles would vary slightly between PRE and PLA at baseline. An additional limiting factor was that several participants, after receiving a NASH diagnosis, made immediate lifestyle changes and lost significant amounts of weight prior to randomization. Weight loss recidivism throughout the intervention may have masked potential treatment effects. Similarly, it is known that energy intake and physical activity patterns can elicit improvements in metabolic parameters of NAFLD [4]. To measure and control for these variables, we utilized 3-day food records and the GLTEQ to determine whether energy intake and physical activity may have influenced our results. Although energy intake and physical activity remained similar between PLA and PRE throughout the study, given the limited reliability of these tools [44,45], we cannot exclude the possibility that differential energy intake and physical activity patterns between groups may have influenced our results. Additionally, due to variability in bowel patterns amongst study participants it was not possible to standardize stool sample collection periods to time of day. Given evidence that gut microbiota composition and activity is under diurnal influence [46], the random stool collection times may have affected gut microbiota composition and metabolites.

In summary, in a pilot clinical trial designed to examine the effectiveness of oligofructose for treating NASH, we demonstrated that oligofructose supplementation improves histological measures of steatosis and overall disease activity score. With a limited number of treatment options available for NASH, gut microbiota-focused interventions using prebiotics offer a safe and sustainable means to manage this exigent disease. Larger scale clinical trials are warranted to further elucidate the potential of prebiotics to treat NASH alone or as an adjunct to other therapies.

**Conflict of Interest**

MRB, JAP, HRR, PC, KPR, CSP, SJ, and MR declare no conflict of interest.RAR previously held a research grant from Beneo-Orafti, Inc., manufacturer of Orafti P95, for a project unrelated to this study.

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**Figure Legends**

**Fig. 1** Flow diagram of participant recruitment, randomization, and analysis.

**Fig. 2** Change in liver biopsy NAS scores after PRE and PLA intervention. Data represented as mean ± SEM. n=8 PRE, n=5 PLA. Wilcoxon signed rank test was used to assess within-group differences and Mann Whitney U test was used to assess between-group differences. **#**indicates P<0.05 between PLA and PRE; **\***indicates P<0.05 within-group change. NAS, Non-alcoholic fatty liver activity score; PLA, placebo; PRE, prebiotic.

**Fig. 3** Gut microbiota in PRE versus PLA over 36 weeks. A) Phyla-level 16S rRNA relative abundance, B) Beta-diversity C) Fecal *Bifidobacterium*, D) Fecal *Clostridium* cluster I, E) Fecal *C.* cluster XI 16S rRNA gene copies represented as mean ± SEM. n=8 PRE, n=6 PLA. NMDS, non-metric multidimensional scaling; PLA, placebo; PRE, prebiotic

Figure 1

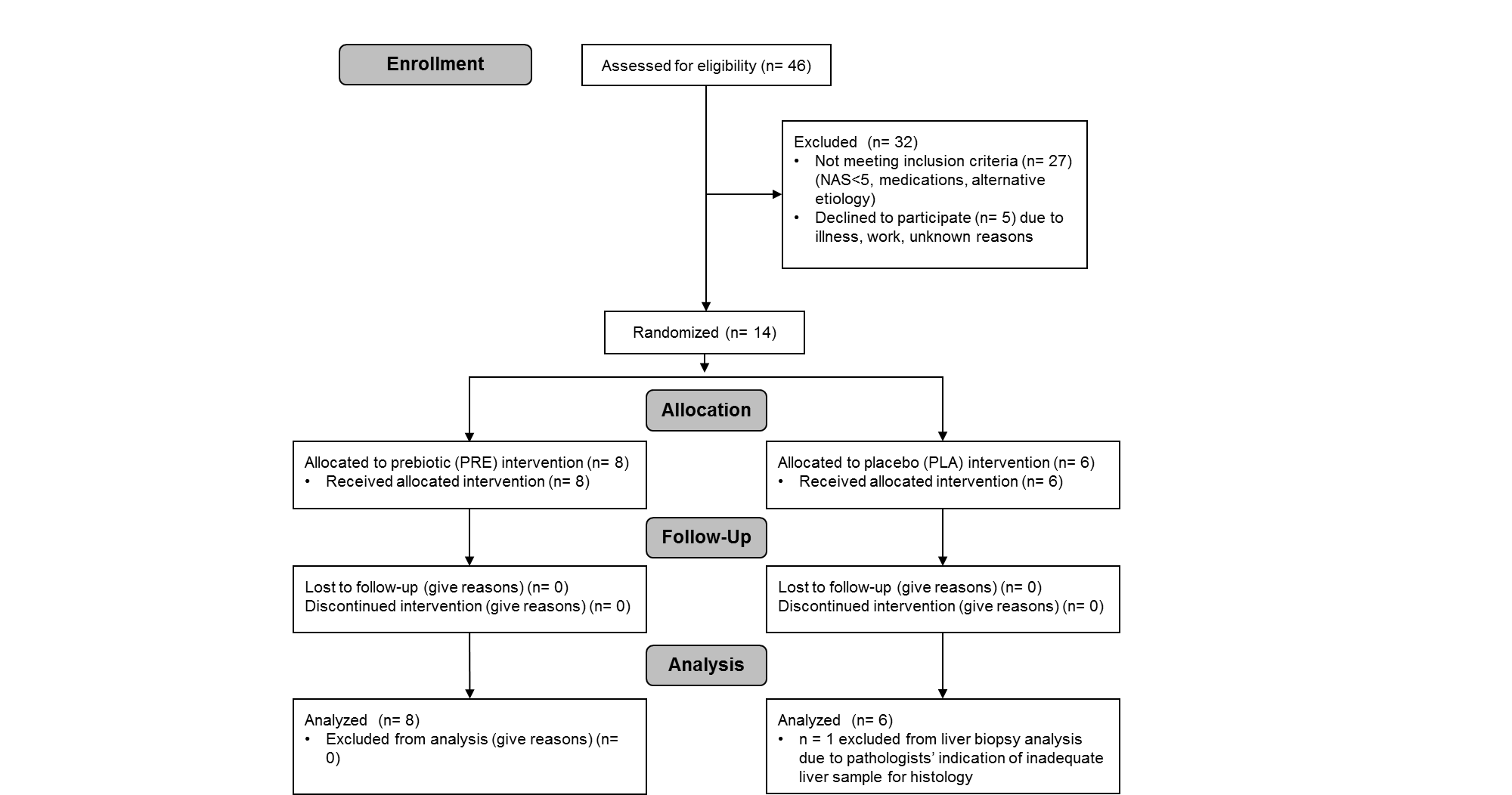


Figure 2



Figure 3



Table 1 Anthropometrics in the PRE and PLA groups over 36 weeks

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Week | | | | P-value | | |
| Measure | Treatment | 0 | 12 | 24 | 36 | Week | Treatment | Interaction |
| Weight | PRE | 101.3 ± 11.4 | 100.7 ± 11.3 | 101.6 ± 11.8 | 101.2 ± 12.2 | 0.849 | 0.949 | 0.998 |
| PLA | 102.1 ± 8.1 | 101.8 ± 8.2 | 102.5 ± 8.3 | 102.3 ± 8.3 |
| BMI, kg/m2 | PRE | 33.7 ± 3.0 | 33.5 ± 2.9 | 33.7 ± 3.0 | 33.6 ± 3.2 | 0.977 | 0.770 | 0.977 |
| PLA | 34.8 ± 2.2 | 34.7 ± 2.2 | 35.0 ± 2.2 | 34.9 ± 2.3 |
| Waist circumference, cm | PRE | 111.5 ± 8.2 | 110.5 ± 8.1 | 111.0 ± 8.3 | 109.1 ± 8.0 | 0.274 | 0.752 | 0.330 |
| PLA | 115.6 ± 6.6 | 113.3 ± 6.7 | 112.6 ± 5.9 | 114.5 ± 5.9 |
| Fat free mass, kg | PRE | 69.3 ± 7.5 | 69.1 ± 7.3 |  | 68.0 ± 7.2 | 0.067 | 0.773 | 0.718 |
| PLA | 66.2 ± 4.6 | 66.3 ± 4.4 | 65.7 ± 4.4 |
| Adipose mass, kg | PRE | 31.9 ± 4.9 | 31.3 ± 4.9 | 32.8 ± 5.9 | 0.409 | 0.653 | 0.893 |
| PLA | 35.6 ± 6.2 | 35.5 ± 6.3 | 36.2 ± 6.5 |
| Body fat, % | PRE | 31.0 ± 2.3 | 30.7 ± 2.4 | 31.6 ± 2.6 | 0.254 | 0.460 | 0.964 |
| PLA | 34.2 ± 3.7 | 34.0 ± 3.7 | 34.7 ± 3.7 |

Data represented as mean ± SEM. n=8 PRE, n=6 PLA. Mixed design (split-plot) repeated measures ANOVA performed to determine main effect of week, treatment, and their interaction. BMI, body mass index; PLA, placebo; PRE, prebiotic.

**Table 2** Liver function tests, inflammatory markers, and glycemic control indices

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | Week | | | | | P-value | | | | |
| Measure | Treatment | | 0 | | 12 | 24 | 36 | Time | Treatment | Interaction | | |
| **Liver function tests** | | | | | | | | | | | | |
| ALT, U/L | | PRE | | 80.4 ± 7.9 | 91.8 ± 19.5 | 75.3 ± 13.1 | 68.1 ± 14.0 | 0.883 | 0.222 | | 0.479 | |
| PLA | | 60.5 ± 14.0 | 51.8 ± 9.6 | 62.7 ± 17.2 | 59.3 ± 14.2 |
| GGT, U/L | | PRE | | 60.5 ± 10.2 | 62.4 ± 9.6 | 62.0 ± 12.4 | 62.3 ± 14.9 | 0.979 | 0.201 | | 0.659 | |
| PLA | | 44.5 ± 8.9 | 41.7 ± 6.4 | 41.3 ± 7.2 | 44.7 ± 7.2 |
| ALP,U/L | | PRE | | 75.4 ± 7.5 | 80.9 ± 7.2 | 81.3 ± 8.6 | 78.8 ± 7.8 | 0.396 | 0.188 | | 0.068 | |
| PLA | | 64.2 ± 7.6 | 62.5 ± 8.0 | 63.2 ± 7.8 | 64.3 ± 7.9 |
| PLA | | 1.12 ± 0.09 | 1.14 ± 0.09 | 1.18 ± 0.09 | 1.12 ± 0.08 |
| **Inflammatory markers** | | | | | | | | | | | | |
| TNF-α, pmol/L | | PRE | | 0.460 ± 0.100 | 0.391 ± 0.087 | 0.416 ± 0.063 | 0.390 ± 0.064 | 0.850 | 0.701 | | | 0.589 |
| PLA | | 0.447 ± 0.039 | 0.468 ± 0.046 | 0.440 ± 0.049 | 0.448 ± 0.041 |
| Il-6, pmol/L | | PRE | | 0.366 ± 0.172 | 0.267 ± 0.295 | 0.269 ± 0.080 | 0.211 ± 0.054 | 0.530 | 0.894 | | | 0.387 |
| PLA | | 0.214 ± 0.047 | 0.165 ± 0.046 | 0.329 ± 0.180 | 0.347 ± 0.164 |
| LPS, EU/mL | | PRE | | 9.73 ± 1.63 | 8.66 ± 0.91 | 9.79 ± 1.64 | 8.03 ± 0.80 | 0.752 | 0.269 | | | 0.164 |
| PLA | | 9.78 ± 1.86 | 10.63 ± 1.70 | 11.63 ± 2.14 | 12.86 ± 1.89 |
| **Glycemic control indices** | | | | | | | | | | | | |
| Fasting glucose, mmol/L | | PRE | | 4.99 ± 0.18\* | 5.04 ± 0.28 | 5.44 ± 0.32 | 5.61 ± 0.59 | 0.619 | **0.022** | | | 0.502 |
| PLA | | 7.02 ± 0.91 | 7.68 ± 1.13 | 6.90 ± 0.96 | 7.28 ± 0.64 |
| Fasting insulin, mU/L | | PRE | | 48.6 ± 9.8 | 51.0 ± 8.4 | 51.3 ± 9.8 | 51.8 ± 9.6 | 0.318 | 0.634 | | | 0.771 |
| PLA | | 37.9 ± 6.3 | 47.8 ± 9.3 | 46.0 ± 7.8 | 46.9 ± 10.5 |
| HOMA2-IR | | PRE | | 5.01 ± 0.97 | 5.27 ± 0.84 | 5.41 ± 0.99 | 5.51 ± 1.02 | 0.262 | 0.878 | | | 0.755 |
| PLA | | 4.38 ± 0.77 | 5.58 ± 1.17 | 5.20 ± 0.94 | 5.27 ± 1.03 |
| Matsuda Index\* | | PRE | | 1.41 ± 0.36 | 0.99 ± 0.16 | 1.21 ± 0.35 | 1.46 ± 0.38 | 0.722 | 0.941 | | | 0.415 |
| PLA | | 1.43 ± 0.48 | 1.37 ± 0.61 | 1.16 ± 0.28 | 1.03 ± 0.24 |

Data represented as mean ± SEM. n=8 PRE, n=6 PLA; \*n=8 PRE, n=5 PLA. Mixed design (split-plot) repeated measures ANOVA performed to determine main effect of week, treatment, and their interaction. \*indicates P<0.05 between PRE and PLA at baseline. ALT, alanine aminotransferase; GTT, γ-glutamyl transferase; HOMA2-IR, homeostasis model assessment of insulin resistance; IL, interleukin; LPS, lipopolysaccharide; PLA, placebo; PRE, prebiotic; TNF-α, tumor necrosis factor-α

Table 3 VAS evaluation of PRE versus PLA dietary intervention

|  |  |  |  |
| --- | --- | --- | --- |
|  | PRE | PLA | P-value |
| **Acceptability** | | | |
| Would you continue to take the supplements now that the study is complete? Not at all (0) - Yes, often (100) | 86.9 ± 7.2 | 90.0 ± 5.6 | 0.950 |
| If the supplements were demonstrated to improve your health, would you continue to take the supplements? Not at all (0) - Yes, often (100) | 94.3 ± 2.4 | 94.5 ± 3.6 | 0.852 |
| **Convenience** | | | |
| How convenient were the supplements? Not at all (0) – Extremely (100) | 71.1 ± 11.7 | 78.8 ± 6.3 | 0.950 |
| How difficult was it to adhere to taking supplement every day? Not difficult (0) - Very difficult (100) | 12.6 ± 4.0 | 20.5 ± 7.6 | 0.491 |
| **Tolerability** | | | |
| How often did you experience negative side effects (flatulence, abdominal discomfort, etc.) that would prevent you from using the supplements? Never (0) – Often 100) | 20.9 ± 9.4 | 31.8 ± 14.2 | 0.852 |
| How often did you experience positive side effects that would encourage you to use the supplements? Never (0) – Often (100) | 52.4 ± 10.8 | 24.8 ± 15.8 | 0.181 |
| **Hunger** | | | |
| Did the supplements decrease cravings for other foods? Not at all (0) – Often (100) | 20.3 ± 6.1 | 8.2 ± 4.6 | 0.142 |
| Did the supplements enhance your feelings of fullness throughout the day? Not at all (0) – Often (100) | 39.8 ± 9.4 | 13.0 ± 6.9 | 0.059 |

Data represented as mean ± SEM; n=8 PRE, n=6 PLA. Participants marked answers along a 100mm continuum. Mann Whitney U test was used to assess between-group differences. PLA, placebo; PRE, prebiotic; VAS, visual analogue scale