

Accepted Manuscript

Prebiotic Reduces Body Fat and Alters Intestinal Microbiota in Children With Overweight or Obesity

Alissa C. Nicolucci, Megan P. Hume, Inés Martínez, Shyamchand Mayengbam, Jens Walter, Raylene A. Reimer

PII: S0016-5085(17)35698-6
DOI: [10.1053/j.gastro.2017.05.055](https://doi.org/10.1053/j.gastro.2017.05.055)
Reference: YGAST 61231

To appear in: *Gastroenterology*
Accepted Date: 30 May 2017

Please cite this article as: Nicolucci AC, Hume MP, Martínez I, Mayengbam S, Walter J, Reimer RA, Prebiotic Reduces Body Fat and Alters Intestinal Microbiota in Children With Overweight or Obesity, *Gastroenterology* (2017), doi: 10.1053/j.gastro.2017.05.055.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Title Prebiotic Reduces Body Fat and Alters Intestinal Microbiota in Children With Overweight or Obesity

Short Title Prebiotic and microbiota in pediatric obesity

Authors Alissa C. Nicolucci¹, Megan P. Hume¹, Inés Martínez², Shyamchand Mayengbam¹, Jens Walter^{2,3}, Raylene A. Reimer^{1,4*}

Author Affiliations ¹Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada

²Department of Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada

³Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

⁴Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada

Grant Support This work was supported by grants from the BMO Financial Group Endowed Research Fund in Healthy Living, Alberta Children's Hospital Foundation, Alberta Children's Hospital Research Institute and the Canadian Institutes of Health Research (MOP115076-1). These agencies had no role in study design, data collection, analysis, and interpretation, or manuscript preparation. ACN and MPH received scholarship funding from the Alberta Children's Hospital Research Institute. ACN received scholarship funding from the Canadian Institutes of Health Research. SM is funded through an Eyes High Postdoctoral Fellowship and an Alberta-Innovates Health Solutions Postdoctoral Fellowship.

Abbreviations A:G – Android to gynoid fat ratio, ANCOVA – Analysis of covariance, ANOSIM – Analysis of similarities, BMI- body mass index, CA – cholic acid, CDCA – chenodeoxycholic acid, CRP – C-reactive protein, DXA – dual-energy x-ray absorptiometry, FBA – fecal bile acid, HOMA2-IR - homeostatic model assessment for insulin resistance 2, IBS – irritable bowel syndrome, IL – interleukin, LPS – lipopolysaccharide, NMDS – nonmetric multidimensional scaling, OI – oligofructose- enriched inulin, OTU – operational taxonomic unit, qPCR – quantitative PCR, T2D- type 2 diabetes

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

Disclosures ACN, MPH, IM, SM and JW declare no conflict of interest. RAR previously held funding from Beneo, manufacturer of oligofructose-enriched inulin, for a project not related to the current work.

Author Contributions RAR conceived the study. ACN and MPH carried out the study and acquired the data. ACN, MPH, IM, SM and JW analysed and interpreted the data. ACN drafted

the manuscript. ACN, IM, JW and RAR critically revised the manuscript for important intellectual content. RAR received funding and had primary responsibility as the study supervisor.

Word Count: 6645

ABSTRACT

Background & Aims: It might be possible to manipulate the intestinal microbiota with prebiotics or other agents to prevent or treat obesity. However, little is known about the ability of prebiotics to specifically modify gut microbiota in children with overweight/obesity or reduce body weight. We performed a randomized controlled trial to study the effects of prebiotics on body composition, markers of inflammation, bile acids in fecal samples, and composition of the intestinal microbiota in children with overweight or obesity.

Methods: We performed a single-center, double-blind, placebo-controlled, trial of 2 separate cohorts (March 2014 and August 2014) at the University of Calgary in Canada. Participants included children, 7 – 12 years old, with overweight or obesity (>85th percentile of body mass index) but otherwise healthy. Participants were randomly assigned to groups given either oligofructose-enriched inulin (OI, 8 g/day; n=22) or maltodextrin placebo (isocaloric dose, controls; n=20) once daily for 16 weeks. Fat mass and lean mass were measured using dual-energy-x-ray absorptiometry. Height, weight, and waist circumference were measured at baseline and every 4 weeks thereafter. Blood samples were collected at baseline and 16 weeks, and analyzed for lipids, cytokines, lipopolysaccharide, and insulin. Fecal samples were collected at baseline and 16 weeks; bile acids were profiled using high-performance liquid chromatography and the composition of the microbiota was analyzed by 16S rRNA sequencing and quantitative PCR. The primary outcome was change in percent body fat from baseline to 16 weeks.

Results: After 16 weeks, children who consumed OI had significant decreases in body weight z-score (decrease of 3.1%), percent body fat (decrease of 2.4%), and percent trunk fat (decrease of 3.8%) compared to children given placebo (increase of 0.5%, increase of 0.05%, and decrease of 0.3%, respectively). Children who consumed OI also had a significant reduction in level of interleukin 6 (IL6) from baseline (decrease of 15%) compared with the placebo group (increase

in 25%). There was a significant decrease in serum triglycerides (decrease of 19%) in the OI group. Quantitative PCR showed a significant increase in *Bifidobacterium* spp. in the OI group compared with controls. 16S rRNA sequencing revealed significant increases in species of the genus *Bifidobacterium* and decreases in *Bacteroides vulgatus* within the group who consumed OI. In fecal samples, levels of primary bile acids increased in the placebo group but not in the OI group over the 16-week study period.

Conclusions: In a placebo-controlled, randomized trial, we found a prebiotic (OI) to selectively alter the intestinal microbiota and significantly reduce body weight z-score, percent body fat, percent trunk fat, and serum level of IL6 in children with overweight or obesity.

Clinicaltrials.gov no: NCT02125955.

KEY WORDS: inulin-type fructans; pediatric obesity; BMI; adiposity

The largest community of microbes in the human microbiota reside in the gut and through a symbiotic relationship with the host, play a role in maintaining health and metabolic homeostasis including the production of a diverse array of metabolites. Dysbiosis is associated with the promotion or aggravation of chronic metabolic diseases including obesity and type 2 diabetes (T2D)¹. One trigger for metabolic disease relates to the gut microbiota's role in modulating inflammation whereby elevated circulating lipopolysaccharide (LPS), which is exacerbated by a high-fat or high-fructose diet, induces a low-grade inflammatory state termed metabolic endotoxemia²⁻⁴. A shift in metabolite production is also observed with dysbiosis, this is particularly true for fecal bile acids (FBA) which require the gut microbiota for transformation⁵. From a clinical stand point there is great interest in determining if modulating the gut microbiota is a viable strategy to manage obesity and improve metabolic health.

Consumption of prebiotics, which are non-digestible food ingredients that are utilized by gut microorganisms and beneficially affect host physiology, is one such strategy^{6,7}. Microbial shifts in response to prebiotic intake have largely centered on changes in *Bifidobacterium* and *Lactobacillus*, two common genera that may be increased with prebiotics and are associated with their beneficial effects on host health⁶. However, with the understanding that global community structure and microbial diversity is important for intestinal and host health, there is a need to examine broader microbial changes that occur in response to prebiotics, for example with sequencing approaches⁷. This approach is lacking in studies with children.

In a systematic review of clinical trials, prebiotic intake was associated with a significant improvement in satiety, postprandial glucose and insulin concentrations in adult subjects⁸. Consumption of an inulin/oligofructose blend has also been shown to increase *Bifidobacterium* spp. and *Faecalibacterium prausnitzii*, which both negatively correlated with LPS and

Bifidobacterium spp. negatively correlated with percent fat mass and low-density lipoprotein cholesterol^{9,10}. These promising outcomes in adults justify the assessment of prebiotics as a dietary intervention to modulate gut microbiota and metabolic outcomes in pediatric obesity.

Excess weight in childhood tends to persist into adulthood and is an early risk factor for obesity-associated morbidity and mortality, highlighting the importance of early intervention¹¹. The potential for prebiotics to influence body weight in children was suggested by the slowed rate of weight gain observed in a trial assessing combined prebiotic and calcium intake in non-obese healthy children^{12,13}. To date, however, there is no research assessing the totality of changes in gut microbiota in children with overweight and obesity with prebiotic intervention. There is also limited research assessing global microbial composition of children with overweight and obesity with or without an intervention. Therefore, our objective was to assess the effect of prebiotic supplementation on gut microbiota, FBAs and associated metabolic outcomes (body composition, serum inflammatory markers, lipid profile and fasting glucose and insulin concentrations) in otherwise healthy children with overweight and obesity.

MATERIALS AND METHODS

Subjects

Male and female children, aged 7-12 years with overweight or obesity ($\geq 85^{\text{th}}$ body mass index (BMI)-percentile) were voluntarily recruited from Calgary, Canada. This was a single centre, double-blind, placebo-controlled trial conducted in two separate cohorts (March 2014 and August 2014) at the University of Calgary. Following screening, subjects were randomly assigned using computer generated numbers (and stratified according to age, sex and BMI) to either prebiotic oligofructose-enriched inulin (OI) or placebo control maltodextrin for 16 weeks. The randomization was performed by an investigator that did not interact with the subjects, and

one research assistant was responsible for all product distribution. Randomization sequences were not revealed to the study staff. Subjects and research staff were blinded to the treatments which were provided in identical foil packets. Parents/care givers completed a health and lifestyle questionnaire on behalf of the subjects to assess eligibility. Eligible subjects were otherwise healthy children with $\geq 85^{\text{th}}$ BMI-percentile at a Tanner developmental stage ≤ 3 (assessed by physical exam by a pediatric endocrinologist from the Alberta Children's Hospital, Calgary, Canada). Exclusion criteria included type 1 or 2 diabetes, liver disease, cardiovascular abnormalities, supplement or medication use influencing appetite, weight or metabolism, currently following a weight loss diet, > 3 kg weight loss 12 weeks prior to the initial test day, extreme changes in exercise intensity 4 weeks prior, or antibiotic use < 3 months prior.

This study, which was powered on the primary objective of reduction in percent body fat with 80 percent power and $\alpha = 0.05$, required a minimum of 18 subjects per group¹⁴. An additional 4 subjects were added per group to compensate for a potential 20% drop out rate⁸. Ethics approval was received from the Conjoint Health Research Ethics Board at the University of Calgary, REB13-0975. Written and informed consent was provided by the parents and verbal assent was provided by the subjects prior to the initial test day.

Dietary intervention

Subjects were randomized to consume either 8 g/day (13.2 kcal/d) of OI, (Synergy1®; BENEIO GmbH, Mannheim, Germany) or an isocaloric dose of 3.3 g/day of maltodextrin placebo (Agenamalt 20.222, Agrana Starch, Konstanz, Germany). Maltodextrin has a similar taste and appearance to Synergy1® and has been used previously in prebiotic trials.⁸ The prebiotic and placebo were consumed as a powder and provided to participants in pre-weighed individual packets. Participants and their parent(s) were instructed to dissolve an entire packet in

250 ml of water in a provided reusable water bottle. They were instructed to consume half the dose for the first 2 weeks, to promote adaptation and mitigate gastrointestinal symptoms, and the full dose for the remaining 14 weeks, 15-20 minutes prior to their evening meal. Empty and unused packets were returned to measure compliance. Our objective was to examine the effects of the prebiotic supplementation independent of any other lifestyle changes therefore subjects purchased their own food, were instructed to eat until comfortably full and maintain their usual level of physical activity. An informal interview was conducted at the end of the study to assess if subjects and their parents remained blinded throughout the study.

Physical characteristics and body composition

Fat mass and lean mass were measured using whole-body dual-energy-x-ray absorptiometry (DXA) (Hologic QDR 4500, Hologic, Inc., Bedford, MA, USA). Android and gynoid fat was estimated using the Hologic QDR software according to Arnberg et al.¹⁵ The android to gynoid fat ratio (A:G) was calculated as [android fat mass/gynoid fat mass]. Height, weight and waist circumference were measured in duplicate at baseline and every 4 weeks thereafter. Height and weight z-scores were calculated using the Baylor College of Medicine-Body Composition Laboratory: Pediatric Body Composition Reference Charts online calculator¹⁶. To track physical activity, subjects completed the Godin's Leisure-Time Exercise Questionnaire¹⁷ at the initial, mid and final test days.

Blood analysis

A fasted blood sample was obtained at the baseline and final test days. Serum lipids were analyzed by Calgary Lab Services (Calgary, Canada). Serum inflammatory cytokines were quantified using Human Adipokine Milliplex kits (Millipore, St. Charles, MO, USA) and Luminex instrument at Eve Technologies (Calgary, AB, Canada). Plasma LPS was measured

using the Pyro-Gene Recombinant Factor C Endotoxin Detection assay (Lonza Group Ltd., Basel, Switzerland) and fasted plasma glucose using the Glucose Trinder assay (Stanbio Laboratory, Boerne, TX, USA). Fasting insulin was quantified using a Human Insulin ELISA kit (Millipore) and insulin resistance estimated using the homeostatic model assessment for insulin resistance 2 (HOMA2-IR)¹⁸.

Fecal bile acid analysis

Fecal bile acids were profiled using high-performance liquid chromatography¹⁹. Briefly, lyophilized, powdered fecal samples (10-20 mg) were suspended in water (250 μ L) and heated for 10 min at 90°C. Samples were cooled then incubated for 16 h at 37°C after adding 250 μ L of sodium acetate buffer (100 mM, pH 5.6) containing 15 units of cholyglycine hydrolase and 150 units of sulfatase. Isopropanol (500 μ L) and 1 M NaOH (100 μ L) were then added and alkaline hydrolysis was performed by incubating 2.5 h at 60°C. An internal standard (nordeoxycholic acid, 50 nM) and 3 mL of 0.1 M NaOH was added and FBAs were extracted through ultrasonication for 1 h. After centrifugation, the supernatant was collected and cleaned using a Sep Pak tC18 cartridge where the FBAs were eluted with 6 mL of methanol. The eluate was dried under Speedvac at 40°C. The unconjugated FBAs were derivatized by adding 150 μ L of triethylamine (10 μ L/mL) and 2-acetobromophenone (12 mg/mL) to their 24-phenacyl esters under ultrasonication for 1.5 h at 50°C. The derivatized FBAs were further cleaned using a Sep-Pak silica cartridge and the eluate was dried under Speedvac at 30°C. The derivatized samples were suspended in 82% methanol and filtered through a 3 kDa centrifuge filter before injecting into the HPLC. Individual BA 24-phenacyl esters were detected at $\lambda_{254\text{nm}}$.

Gut bacterial community profiling – 16S rRNA quantitative PCR

Subjects and parent(s) were instructed to collect a stool sample preferably the evening before but up to 3 days prior to the baseline and final test days using a stool collection kit. Stool was placed in a sterile conical tube and stored in a biohazard bag in the participant's home freezer. Samples were brought to the laboratory on ice and stored at -80°C until analysis. Total bacterial DNA was extracted using FastDNA Spin Kit for Feces (MP Biomedicals, Lachine, QC, Canada) and quantified using the Nanodrop 2000 (Thermo Fisher Scientific Inc., Waltham, MA). All samples were diluted to 4 ng/μL prior to storage at -30°C. Amplification and detection was conducted in 96-well plates with SYBR Green qPCR Master Mix (BioRad, Hercules, CA) according to our previous work²⁰ using group specific primers²¹.

Gut bacterial community profiling – 16S rRNA Illumina sequencing

Ethanol precipitation was performed on extracted bacterial DNA to ensure a purified sample. Quantification was performed using Qubit dsDNA assay (Life Technologies, Grand Island, NY) and diluted to 5 ng/μL working concentration. Microbial profiling was conducted using the Illumina MiSeq platform, according to the Illumina 16S Metagenomics Sequencing Library Preparation protocol and in accordance with our previous work²² (Centre for Health Genomics and Informatics, Calgary, AB, Canada). Primary PCRs amplified the V3-V4 region of the 16S rRNA gene and secondary PCRs attached dual indices to amplified regions with manufacturer recommended primers²². Sequencing was performed with dual indexed paired 300 bps. Results were approximately 20 million total reads.

Gut bacterial community analysis - 16S rRNA Illumina sequencing

Due to poor merging between the primers, the V3 region was selected for the analysis as it consisted of higher quality sequences. Microbial community analysis was performed as described in Krumbeck et al.²³ Sequences were trimmed to 250 nts and filtered using the

FASTX-toolkit. Chimeras were removed and operational taxonomic units (OTUs) with 98% homology were generated using USEARCH. Sequences were classified from phylum to genus level using the Ribosomal Database Project MultiClassifier. Alpha and beta-diversity metrics were calculated in QIIME using rarefied data to control for the number of sequences in each sample. All taxonomic data was calculated as proportions of sequences based on the total number of sequences for each sample.

Statistical analysis – Biological and qPCR outcomes

Data is presented as mean \pm SEM. Analysis was performed on an intent-to-treat basis, regardless of subject compliance or completion. Cases with missing outcomes were excluded from analysis for that outcome. Normality was verified for each outcome and corresponding non-parametric tests were conducted on outcomes with a skewed distribution. Parametric tests were used to compare baseline measurements (independent t-test), between-group differences using mean differences from the two time points (independent t-test) and within-group differences (dependent t-test). Outcomes were further analyzed using an analysis of covariance (ANCOVA) assessing for sex, age and BMI as potential covariates. Height, body weight and waist circumference which were measured multiple times were analyzed using a mixed model repeated measures ANOVA with Bonferroni adjustment.

Statistical analysis – 16S rRNA Illumina sequencing

Data is presented as mean \pm SD. All statistical analysis was preformed using R version 3.2.2²⁴. Wilcoxon signed-rank tests were used to analyze within group differences for microbial groups and alpha-diversity. Between group differences were evaluated using linear mixed-effect model with package “lme4” assessing the interaction between treatment groups and time²⁵. Beta-diversity was assessed based on Bray-Curtis distances and significance was determined using an

analysis of similarities (ANOSIM) with the “vegan” package²⁶. To account for multiple comparisons an FDR correction was applied with the “fdrtool” package²⁷. For corrected p values significance was set at < 0.2 .

All authors had access to study data and reviewed and approved the final manuscript.

RESULTS

Subject characteristics

A total of 42 subjects consented to participate in the study of which 22 were randomized to the prebiotic group and 20 to the placebo. A total of 4 subjects withdrew for personal reasons (time constraints) or otherwise not specified (Supplementary Figure 1). Therefore, a total of 38 children, 20 in the prebiotic group and 18 in the control group, completed the study (90% retention). There were no significant differences in baseline characteristics between the groups (Table 1). A total of 81.8% of participants were Caucasian and 18.2% classified as “Other” represented by Black and Hispanic.

Improvements in anthropometry and body composition with prebiotic

Prebiotic consumption slowed weight gain compared to placebo. Although absolute body weight increased in both groups over 16 weeks, the increase was significantly higher in placebo (2.4-fold greater weight gain) compared to prebiotic (Table 2). Age and sex-specific analysis of body weight showed significant decreases in body weight z -score within the prebiotic group ($P=0.006$). The interaction of treatment and time significantly influenced BMI ($P=0.009$) whereby there was no change in BMI within the prebiotic group compared to baseline, but at all four time points compared to baseline, BMI significantly increased in the placebo group (Supplementary Figure 2). Percent total body fat was significantly lower with OI compared to placebo ($P=0.005$; Figure 1). Both groups had a significant increase in lean mass. Regional body

fat assessment showed significant decreases in percent trunk fat within the prebiotic group ($P=0.019$) and significant differences between the groups ($P=0.029$). Percent android fat tended to be reduced in prebiotic versus placebo ($P=0.055$).

Prebiotics induced marginal changes in systemic inflammation

There was little change in the serum inflammatory profile within the prebiotic and placebo groups (Supplementary Table 1). Although there was a 31% decrease in serum C-reactive protein (CRP) in the prebiotic group and an 8% increase in the placebo group, this was not significantly different. Between-group analysis did show a significant reduction in interleukin (IL)-6 from baseline with OI whereas placebo increased ($P=0.01$). There was a trend ($P=0.088$) for LPS to be decreased ($1.9\times$) with OI intake and increased with placebo ($1.4\times$).

Metabolic outcomes

There was a significant decrease in serum triglycerides within the prebiotic group but no between-group differences in lipid profile (Supplementary Table 2). There were no differences in fasting glucose, insulin or HOMA2-IR within or between groups. At the end of the trial however, four of the subjects in the prebiotic group (3 male, 1 female, baseline BMI 29.0 ± 2.9 kg/m², baseline percent total body fat $46.7 \pm 2.2\%$, baseline trunk fat $46.7 \pm 2.2\%$, baseline HOMA2-IR 2.5 ± 0.2), compared to zero in the placebo group, were no longer classified as insulin resistant as defined by HOMA2-IR ($\text{HOMA2-IR} > 2.10$)²⁸.

Fecal bile acids

Primary FBAs, cholic acid (CA) and chenodeoxycholic acid (CDCA) were significantly different between the OI and placebo group after adjusting for age, sex, initial BMI and compliance (Table 3). Within group analysis showed significant increases in both primary FBAs in the placebo group. There were no significant changes in secondary FBAs within or between

treatment groups. The percentage of CDCA to total FBAs (CDCA%) was significantly increased within the placebo group over time and the between group difference showed a higher percentage in placebo versus OI.

Characterization of gut microbial changes

Quantitative analysis of specific taxa (qPCR) showed a within group difference of increased *Bifidobacterium* spp. in prebiotic ($P=0.023$) and decreased *Clostridium* cluster XI ($P=0.044$) in placebo (Supplementary Table 3). A significant between-group difference was seen for *Bifidobacterium* spp. with OI intake resulting in significantly higher abundance than placebo at 16 weeks ($P=0.049$). The change in *Bifidobacterium* spp. from baseline was also significantly different between the prebiotic and placebo group ($1.71\% \pm 0.80$ vs. $0.13\% \pm 0.94$, $P=0.049$).

A community-wide analysis with Illumina 16S rRNA sequencing mirrored the outcomes from qPCR (Table 4). OI consumption resulted in a significant bifidogenic response over the 16 weeks within the prebiotic group. Actinobacteria, the only observed phylum level change, significantly increased 1.4 fold ($P=0.008$, FDR=0.217) and at the genus level *Bifidobacterium* abundance significantly increased. Moreover, two OTUs most likely representing *Bifidobacterium adolescentis* (OTU_2169) and *Bifidobacterium longum* (OTU_2403) significantly increased. Between-group analysis highlighted an interaction effect of treatment and time on *Bifidobacterium longum* (OTU_14) although not statistically significant (Supplementary Table 4).

Further analysis of within and between-group differences showed significant changes in bacterial community composition beyond *Bifidobacterium*. Within group analysis showed significant decreases in *Faecalibacterium prausnitzii* (OTU_2516) abundance with prebiotic ($P=0.002$, FDR=0.153) (Table 4). The interaction between treatment and time significantly

influenced two separate OTUs representing *F. prausnitzii* (OTU_2516 and OTU_1938) (Supplementary Table 4). Although no significant changes in the Bacteroidetes phylum or the genus *Bacteroides* were observed, *Bacteroides* sp. (OTU_29) was significantly influenced by the interaction of treatment and time. OTU level analysis of within group differences revealed OI significantly decreased *Bacteroides vulgatus* (OTU_2492, $P=0.005$, FDR=0.155) (Table 4). Significant decreases in OTU_2376 representing *Ruminococcus gauvreauii* were also observed with consumption of OI whereas decreases observed in genus *Ruminococcus* were not statistically significant following adjustment ($P=0.026$, FDR=0.311).

The Shannon and Simpson Index, which were used to assess alpha-diversity, significantly decreased within both groups and no significant change in observed OTUs was detected within either group (Table 4). Beta-diversity, assessed using nonmetric multidimensional scaling (NMDS) plots based on Bray-Curtis distances, revealed differential clustering within the prebiotic group from baseline to 16 weeks determined using an ANOSIM ($P=0.042$) (Supplemental Figure 2). However, there was no appreciable differential clustering of the data observed between the groups or within the placebo group.

Microbial correlations with clinical biomarkers

Spearman's correlation analysis was used to assess the relationship between changes in gut microbial abundance and changes in body composition and biological parameters (Figure 2). When assessing correlations with change in body composition, changes in body weight ($r_s=0.414$, $P=0.012$), fat mass ($r_s=0.358$, $P=0.032$) and BMI ($r_s=0.373$, $P=0.025$) were significantly and positively correlated with changes in OTU_2559 representing *Clostridium clostridioforme* and change in trunk body fat was significantly and positively correlated with change in *Bacteroides vulgatus* (OTU_2492) ($r_s=0.494$, $P=0.002$) and change in *bacterium mpn-*

isolate (OTU_1554) ($r_s=0.394$, $P=0.017$). Change in OTU_2559 was also significantly and positively correlated with changes in IL-6 ($r_s=0.657$, $P=0.0001$) whereas change in serum triglycerides was significantly and positively correlated with change in *Ruminococcus gauvreauii* (OTU_2376) ($r_s=0.479$, $P=0.005$).

Side effects and compliance

No gastrointestinal side effects were experienced by 70% of participants in the prebiotic group and 61.1% in the placebo. A mild increase in flatulence and bloating was experienced by 25% and 27.8% of subjects in prebiotic and placebo respectively. A moderate increase in flatulence and bloating was reported by 5% and 11.1% of subjects in prebiotic and placebo respectively. During the informal interview to assess blinding, 50% of the prebiotic group and 72.2% of the placebo group were able to correctly guess their grouping. There was 87% and 91% compliance in the prebiotics and placebo group respectively.

DISCUSSION

This is the first randomized controlled study to assess the totality of changes in gut microbial composition and FBAs with prebiotic intervention in children with overweight and obesity. The results demonstrate that OI consumption normalizes childhood weight gain, reduces whole body and trunk body fat, modifies primary FBAs and selectively alters gut microbiota.

Although weight loss is a key outcome in obesity interventions, pediatric trials must consider the confounding effects of growth. One other trial with prebiotics in youth²⁹ did not observe a reduction in absolute body weight with OI which is consistent with our findings. However, height and weight are expected to increase linearly in children 6-10 years old with little difference between the sexes, in particular body weight is expected to increase 2-3 kg annually³⁰. Based on our 4-month intervention data, the annual projected body weight increase in

the prebiotic group would be 3 kg, within the expected range, whereas the projected increase in the placebo group was 8 kg, almost triple the expected yearly increase. The normalization of absolute body weight gain with OI is important as it allowed children to meet and not exceed expected growth trends²⁹. This normalization may be attributed in part to the improved appetite control we previously demonstrated in the OI group³¹.

In the present study, the reduction in percent body fat observed in the subjects consuming OI was similarly observed by Abrams et al.¹³ as a reduction in total fat mass in normal weight and overweight children consuming 8 g of OI with supplemental calcium for a year. Important from a metabolic health perspective³², percent trunk fat was decreased in our participants consuming OI which was similarly observed in adults with overweight and obesity consuming oligofructose for 12 weeks⁸. The decrease in central adiposity in the present study could explain in part the significant reduction in serum triglycerides observed in the prebiotics group.

A proposed mechanistic link between obesity and its associated comorbidities, such as insulin resistance, is low-grade inflammation. Increased IL-6 and TNF- α are seen in adult obesity³³, while CRP is positively correlated with obesity in children and adults^{33,34}. In healthy, normal weight adults there was no change in cytokines following prebiotic intake, likely because baseline levels were not elevated enough to detect differences³⁵. This is likely the case in our subjects as well given that IL-6 was the only cytokine significantly reduced with prebiotic and greater inflammation is typically needed to see a treatment response^{36,37}. We did observe a trend towards a reduction in metabolic endotoxemia which is consistent with reduced LPS seen with inulin-type fructan intake in otherwise healthy obese adults, healthy normal weight adults and overweight and obese women with T2D^{9,35,38-40}.

Microbial metabolites, such as FBAs, are one potential mechanism through which changes

in gut microbiota composition impact host physiology⁴¹. Increased primary FBAs have been associated with negative clinical outcomes, including diarrhea-prominent irritable bowel syndrome, which was associated with a significant decrease in *Bifidobacterium*⁵. Of relevance to obesity, increased serum levels of the primary BA, CDCA relative to total BAs (CDCA%) was seen in obesity with type 2 diabetes > obesity > overweight > healthy control⁴². There was also a positive correlation between CDCA% and BMI, HbA1c, LDL-cholesterol and triglycerides⁴². In our participants, no change in fecal CDCA% was seen in the OI group (albeit a numerical but not significant decrease) but there was a significant 17% increase in the placebo group over time. It is possible that intake of OI mitigated the natural trajectory of increased primary FBAs seen in the placebo group, in part through increased *Bifidobacterium*⁴³.

Although our current understanding of what constitutes a healthy microbiota is still incomplete, certain genera have been established as primarily beneficial, including species in the genus *Bifidobacterium*⁴⁴. Infant studies highlight the benefits of increased bifidobacteria, with *Bifidobacterium* spp. dominating the gut of breast-fed infants which is associated with a reduced likelihood of overweight and obesity in childhood^{45,46}. Similarly, adults with obesity had a significant reduction in *Bifidobacterium* spp. compared to healthy weight⁴⁷. Our analysis of microbial changes at the genus level showed sequencing results that mirrored those from qPCR. Significant increases in *Bifidobacterium* spp. within the prebiotic group in this study was the only microbial change when assessed with qPCR which was also observed in diverse adult cohorts consuming various prebiotics^{9,10,35}. Sequencing analysis in the present study also showed significant increases in OTUs representing *Bifidobacterium* such as *Bifidobacterium longum* (OTU_2403) which was similarly observed after prebiotic supplementation in women with obesity¹⁰. In the adult human gut, significant increases in *Bifidobacterium* were observed after

>10g/day of short-chain fructooligosaccharides⁴⁸. In our pediatric population, we observed a significant bifidogenic response in the prebiotic group with a lower 8 g/day dose of OI.

The definition of prebiotic is currently a hotly debated issue largely due to advancements in high throughput sequencing showing changes beyond *Bifidobacterium* and *Lactobacillus* and the requirement for selective utilization⁷. In accordance with this debate, changes in several other species and genera were observed with OI consumption, although the number remained limited supporting a selective utilization argument. In the present study a significant decrease in *Bacteroides vulgatus* (OTU_2492) was observed with OI consumption. Importantly this reduction in *B. vulgatus* was correlated with a reduction in percent trunk fat over the 16-week intervention. This positive correlation between *B. vulgatus* and adiposity was also observed in women with obesity after prebiotic intervention⁹. *C. clostridioforme* has been defined as a pathogenic bacteria associated with serious and invasive human infection⁴⁹. In reference to metabolic disease, two metagenome projects observed *C. clostridioforme* significantly enriched in patients with T2D compared to healthy controls^{50,51}. *Clostridium clostridioforme* (OTU_2559) in the present study, decreased in prebiotic versus placebo, and it was significantly positively correlated with changes in different biological and compositional outcomes.

Faecalibacterium prausnitzii is a prominent butyrate-producing bacterium that has been suggested to have an anti-inflammatory role in inflammatory bowel disease and has been negatively correlated with LPS in participants with obesity^{9,52}. In the present study however, *F. prausnitzii* (OTU_2516) significantly decreased with OI consumption. This result is consistent with a cross-sectional study in India showing increased *F. prausnitzii* abundance in children with obesity compared to non-obese children with qPCR⁵³ and more recently with Illumina sequencing showing 20% higher abundance of *F. prausnitzii* in Italian children with obesity

compared to normal-weight⁵⁴, including a positive correlation of BMI z-score with *Faecalibacterium*. Conversely, in adult populations prebiotic significantly increased *F. prausnitzii* abundance^{9,55} and these differences may be due to the cross-feeding interactions between bifidobacteria and *F. prausnitzii*⁵⁶. *In vitro* analysis revealed that the relationship between bifidobacteria and *F. prausnitzii*, in the presence of inulin-type fructans, could be commensal or competitive and this relationship was dependent on the bifidobacterial strain and its capacity for prebiotic degradation. Functional differences between genetic phylotypes of *F. prausnitzii* with different capacities for butyrate production have also been observed with lean individuals having a genetic variant with a more moderate capacity for butyrate production compared to individuals with obesity and T2D⁵⁷. In accordance with this, children with obesity had higher stool concentrations of butyrate compared to normal-weight controls⁵⁸.

There are some limitations to our study including a reduced generalizability of our findings due to the primarily Caucasian and middle to high socioeconomic status of our participants. Our participants were also otherwise healthy overweight and obese children and therefore future studies should also include a pediatric population with greater metabolic dysfunction to more fully understand how genotype and environment affect the relationship between the host and the gut microbiome⁷. Lastly, many children in the study did not have regular bowel movements, therefore our stool collection at baseline and final test days could not be tightly controlled (e.g. time of day, 24 hour collection), which could affect the concentrations of some fecal metabolites such as FBAs.

In conclusion, supplementation with OI improved obesity outcomes in children with overweight/obesity. Importantly, we have shown that OI induced specific gut bacterial shifts compared to placebo. The metabolic and microbial findings from this study provide a foundation

for a larger clinical trial in the pediatric population. Prebiotics are inexpensive and non-invasive and therefore a plausible dietary intervention in the overweight and obese pediatric population.

Acknowledgements The authors would like to thank Kristine Lee, Faculty of Kinesiology, University of Calgary for technical assistance. Shelly Wegener, Dr. Richard Pon, and Dr. Paul Gordon, University Core DNA Services and Alberta Children's Hospital Research Institute (ACHRI) Centre for Health Genomics and Informatics at the University of Calgary for their technical assistance and support with the 16S rRNA sequencing and analysis. This work was supported by grants from the BMO Financial Group Endowed Research Fund in Healthy Living, Alberta Children's Hospital Foundation, Alberta Children's Hospital Research Institute and the Canadian Institutes of Health Research (MOP115076-1). The funding agencies had no role in the design of the study or preparation of this manuscript, and had no influence on the data collection, analysis and interpretation or manuscript publication. The oligofructose-enriched inulin (Synergy1[®]) was provided by Beneo (Mannheim, Germany).

Additional information Supplementary Information accompanies this paper.

REFERENCES

1. Arora T, Backhed F. The gut microbiota and metabolic disease: Current understanding and future perspectives. *J Intern Med* 2016;1–11.
2. Cani PD, Bibiloni R, Knauf C, et al. Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet – Induced Obesity and Diabetes in Mice. *Diabetes* 2008;57:1470–1481.
3. Cani PD, Amar J, Iglesias MA, et al. Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. *Diabetes* 2007;56:1761–1772.
4. Spruss A, Kanuri G, Stahl C, et al. Metformin protects against the development of fructose-induced steatosis in mice: role of the intestinal barrier function. *Lab Invest* 2012;92:1020–1032.
5. Duboc H, Rainteau D, Rajca S, et al. Increase in fecal primary bile acids and dysbiosis in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2012;24.
6. Gibson GR, Scott KP, Rastall RA, et al. Dietary prebiotics : current status and new definition. *Food Sci Technol Bull Funct Foods* 2010;7:1–19.
7. Bindels LB, Delzenne NM, Cani PD, et al. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol* 2015;12:303–310.
8. Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr* 2009;89:1751–1759.
9. Dewulf EM, Cani PD, Claus SP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* 2013;62:1112–1121.
10. Salazar N, Dewulf EM, Neyrinck AM, et al. Inulin-type fructans modulate intestinal *Bifidobacterium* species populations and decrease fecal short-chain fatty acids in obese women. *Clin Nutr* 2015;34:501–507.
11. Weiss R, Caprio S. The metabolic consequences of childhood obesity. *Best Pract Res Clin Endocrinol Metab* 2005;19:405–19.
12. Abrams SA, Griffin IJ, Hawthorne KM, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young

- adolescents. *Am J Clin Nutr* 2005;82:471–476.
13. Abrams SA, Griffin IJ, Hawthorne KM, et al. Effect of prebiotic supplementation and calcium intake on body mass index. *J Pediatr* 2007;151:293–298.
 14. Savoye M, Shaw M, Dziura J, et al. Effects of a Weight Management Program on Body Composition and Metabolic Parameters in Overweight Children. *JAMA* 2007;297:2697–2704.
 15. Arnberg K, Larnkjær A, Michaelsen KF, et al. Central Adiposity and Protein Intake Are Associated with Arterial Stiffness in Overweight Children. *J Nutr* 2012;142:878–885.
 16. USDA/ARS Children’s Nutrition Research Center. Pediatric Body Composition Reference Charts. Available at:
<https://www.bcm.edu/bodycomplab/Flashapps/AllDXArefsChartpage.html>.
 17. Godin G, Shephard RJ. Godin Leisure-Time Exercise Questionnaire. *Med Sci Sports Exerc* 1997;29:S36–S38.
 18. The Oxford Centre for Diabetes Endocrinology and Metabolism. HOMA Calculator. Available at: <https://www.dtu.ox.ac.uk/homacalculator/>.
 19. Kakiyama G, Muto A, Takei H, et al. A simple and accurate HPLC method for fecal bile acid profile in healthy and cirrhotic subjects: validation by GC-MS and LC-MS. *J Lipid Res* 2014;55:978–990.
 20. Bomhof MR, Saha DC, Reid DT, et al. Combined effects of oligofructose and *Bifidobacterium animalis* on gut microbiota and glycemia in obese rats. *Obesity* 2014;22:763–71.
 21. Collins KH, Paul HA, Reimer RA, et al. Relationship between inflammation, the gut microbiota, and metabolic osteoarthritis development: Studies in a rat model. *Osteoarthritis Cartilage* 2015;23:1989–1998.
 22. Bomhof MR, Paul HA, Geuking MB, et al. Improvement in adiposity with oligofructose is modified by antibiotics in obese rats. *FASEB J* 2016;30:fj-201600151R.
 23. Krumbeck JA, Maldonado-Gomez MX, Martínez I, et al. In Vivo Selection To Identify Bacterial Strains with Enhanced Ecological Performance in Synbiotic Applications. *Appl Environ Microbiol* 2015;81:2455–2465.
 24. R Core Team. R: A Language and Environment for Statistical Computing. R Found Stat Comput 2015:Vienna, Austria.

25. Bates D, Mächler M, Bolker B, et al. Fitting Linear Mixed-Effects Models using lme4. *J Stat Softw* 2015;67:1–48.
26. Oksanen J, Blanchet FG, Kindt R, et al. *vegan: Community Ecology Package* R package version 2.3-2. 2015.
27. Strimmer K. *fdrtool: A versatile R package for estimating local and tail area-based false discovery rates*. *Bioinformatics* 2008;24:1461–1462.
28. Manios Y, Moschonis G, Kourlaba G, et al. Prevalence and independent predictors of insulin resistance in children from Crete, Greece: the Children Study. *Diabet Med* 2008;25:65–72.
29. Liber A, Szajewska H. Effect of oligofructose supplementation on body weight in overweight and obese children: a randomised, double-blind, placebo-controlled trial. *Br J Nutr* 2014;112:2068–2074.
30. Malina R. Normal Weight Gain in Growing Children. *Heal Weight J* 1999;13:13–14.
31. Hume MP, Nicolucci AC, Reimer RA. Prebiotic supplementation improves appetite control in children with overweight and obesity: a randomized controlled trial. *Am J Clin Nutr* 2017;105:790-799.
32. Despres J-P, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881–887.
33. Bastard J-P, Jardel C, Bruckert E, et al. Elevated Levels of Interleukin 6 Are Reduced in Serum and Subcutaneous Adipose Tissue of Obese Women after Weight Loss. *J Clin Endocrinol Metab* 2000;85:3338–3342.
34. Ford ES, Galuska DA, Gillespie C, et al. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988-1994. *J Pediatr* 2001;138:486–492.
35. Lecerf J-M, Dépeint F, Clerc E, et al. Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. *Br J Nutr* 2012;108:1847–1858.
36. Varma MC, Kusminski CM, Azharian S, et al. Metabolic endotoxaemia in childhood obesity. *BMC Obes* 2015;3:1.
37. Weiss R, Dziura J, Burgert TS, et al. Obesity and the Metabolic Syndrome in Children and Adolescents. *N Engl J Med* 2004;350:2362–2374.

38. Dehghan P, Pourghassem Gargari B, Asghari Jafar-abadi M. Oligofructose-enriched inulin improves some inflammatory markers and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized controlled clinical trial. *Nutrition* 2014;30:418–423.
39. Dehghan P, Pourghassem Gargari B, Asghari Jafar-Abadi M, et al. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. *Int J Food Sci Nutr* 2014;65:117–123.
40. Parnell JA, Klancic T, Reimer RA. Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity. *Obesity* 2017;25:510–513.
41. Wahlstrom A, Sayin SI, Marschall HU, et al. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. *Cell Metab* 2016;24:41–50.
42. **Yu H, Ni Y**, Bao Y, et al. Chenodeoxycholic Acid as a Potential Prognostic Marker for Roux-en-Y Gastric Bypass in Chinese Obese Patients. *J Clin Endocrinol Metab* 2015;100:4222–4230.
43. Ridlon J, Kang D, Hylemon P, et al. Bile acids and the Gut Microbiome. *Curr Opin Gastroenterol* 2014;30:332–338.
44. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995;126:1401–12.
45. Gioia D Di, Aloisio I, Mazzola G, et al. Bifidobacteria: their impact on gut microbiota composition and their applications as probiotics in infants. *Appl Microbiol Biotechnol* 2013;98:563–77.
46. Kalliomäki M, Collado MC, Salminen S, et al. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008;87:534–538.
47. Schwiertz A, Taras D, Schäfer K, et al. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* 2009;18:190–195.
48. Bounie Y, Vahedi K, Achour L, et al. Short-Chain Fructo-Oligosaccharide Administration Dose-Dependently Increases Fecal Bifidobacteria in Healthy Humans. *J Nutr* 1999;129:113–116.
49. Finegold SM, Song Y, Liu C, et al. *Clostridium clostridioforme*: A mixture of three clinically important species. *Eur J Clin Microbiol Infect Dis* 2005;24:319–324.
50. **Karlsson FH, Tremaroli V**, Nookaew I, et al. Gut metagenome in European women with

- normal, impaired and diabetic glucose control. *Nature* 2013;498:99–103.
51. **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.
 52. **Sokol H, Pigneur B**, Watterlot L, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A* 2008;105:16731–16736.
 53. Balamurugan R, George G, Kabeerdoss J, et al. Quantitative differences in intestinal *Faecalibacterium prausnitzii* in obese Indian children. *Br J Nutr* 2010;103:335–338.
 54. Riva A, Borgo F, Lassandro C, et al. Pediatric obesity is associated with an altered gut microbiota and discordant shifts in Firmicutes populations. *Environ Microbiol* 2017;19:95–105.
 55. Ramirez-Farias C, Slezak K, Fuller Z, et al. Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br J Nutr* 2009;101:541–550.
 56. Moens F, Weckx S, Vuyst L De. Bifidobacterial inulin-type fructan degradation capacity determines cross-feeding interactions between bifidobacteria and *Faecalibacterium prausnitzii*. *Int J Food Microbiol* 2016;231:76–85.
 57. Hippe B, Remely M, Aumueller E, et al. *Faecalibacterium prausnitzii* phylotypes in type two diabetic, obese, and lean control subjects. *Benef Microbes* 2016;7:511–517.
 58. Payne AN, Chassard C, Zimmermann M, et al. The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. *Nutr Diabetes* 2011;1:e12.

Authors names in bold designate shared co-first authorship.

Figure Legends

Figure 1 Change in body composition in the prebiotic (n = 22) and placebo (n = 19) groups over the 16-week intervention represented by mean \pm SEM. * $P < 0.05$ and † $P < 0.01$ with independent t-test between the two groups.

Figure 2 Heat map of the Spearman rank correlations between biological and gut microbial outcomes. Correlations were performed on the change in outcomes over the 16-week intervention. * $P < 0.05$ and † $P < 0.01$. BF, body fat; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; IL, interleukin; TNF α , tumor necrosis factor alpha; UDCA, ursodeoxycholic acid; DCA, deoxycholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; HDCA, hyodeoxycholic acid; LCA, lithocholic acid; FA, Fecal Bile Acids.

Table 1. Participant baseline characteristics by treatment group¹

Characteristics	Prebiotics	Placebo	<i>P</i> value
Sex, Number			
Male	12	12	0.721
Female	10	8	
Age, y	10.4 ± 0.3	10.2 ± 0.4	0.724
Body weight, kg	58.5 ± 3.1	59.6 ± 4.5	0.837
Body weight z-score	2.25 ± 0.12	2.14 ± 0.16	0.573
Height, cm	148.1 ± 2.4	147.1 ± 2.8	0.783
Height z-score	1.31 ± 0.24	0.97 ± 0.22	0.304
BMI, kg/m ²	26.3 ± 0.7	26.9 ± 1.3	0.653
% Total Body Fat	42.5 ± 1.3	41.8 ± 1.2	0.688

¹Values are mean ± SEM, *n*=22 prebiotics and *n*=20 placebo; 1 participant did not attend the initial test day so researcher-measured outcomes are *n*=19 for placebo.

z-score, standard score; BMI, body mass index

Table 2. Changes in anthropometric outcomes in children (7-12 years) with overweight and obesity consuming oligofructose-enriched inulin (prebiotic) or placebo for 16 weeks¹

Outcome	Prebiotics				Placebo				Between groups <i>P</i> value
	Initial	Final	Within group <i>P</i> value	Change	Initial	Final	Within group <i>P</i> value	Change	
Height (cm)	148.1 ± 2.4	150.5 ± 2.4	< 0.001	2.3 ± 0.3	147.1 ± 2.8	149.1 ± 2.9	< 0.001	2.0 ± 0.2	0.354
Height z-score	1.31 ± 0.24	1.37 ± 0.24	0.139	0.06 ± 0.04	0.97 ± 0.22	0.99 ± 0.23	0.481	0.03 ± 0.04	0.509
Body Weight (kg)	58.5 ± 3.1	59.6 ± 3.1	0.009	1.1 ± 0.4	59.6 ± 4.5	62.2 ± 4.8	< 0.001	2.6 ± 0.4	0.009
Body Weight z-score	2.25 ± 0.10	2.18 ± 0.12	0.006	-0.07 ± 0.02	2.14 ± 0.16	2.14 ± 0.16	0.79	0.01 ± 0.02	0.024
Waist-Iliac Crest (cm)	87.3 ± 2.1	92.0 ± 2.2	< 0.001	4.8 ± 0.9	88.1 ± 3.0	93.7 ± 3.5	< 0.001	5.6 ± 0.1	0.520
Waist-Umbilicus (cm)	91.2 ± 2.2	89.2 ± 2.1	0.012	-2.1 ± 0.8	92.6 ± 3.3	90.9 ± 3.2	0.009	-1.7 ± 0.6	0.814
Bone Mineral Density	0.72 ± 0.01	0.73 ± 0.01	0.018	0.01 ± 0.04	0.71 ± 0.01	0.71 ± 0.01	0.116	0.01 ± 0.02	0.592
BMI (kg/m ²)	26.2 ± 0.7	26.0 ± 0.7	0.199	-0.3 ± 0.2	26.9 ± 1.3	27.3 ± 1.3	0.02	0.4 ± 0.2	0.004

¹Values are mean ± SEM, *n*=22 for prebiotic and *n*=19 for placebo. Z-score, standard score; BMI, body mass index.

Table 3. Changes in fecal bile acids assessed using HPLC in children (7-12 years) with overweight and obesity consuming oligofructose-enriched inulin (prebiotic) or placebo for 16 weeks¹

Prebiotics				Placebo			
Outcome ($\mu\text{mol/g}$, dry feces)	Initial	Final	Within group <i>P</i> value	Initial	Final	Within group <i>P</i> value	Between groups <i>P</i> value
Primary Bile Acids							
CA	1.651 \pm 0.500	2.290 \pm 0.949	0.967	1.801 \pm 0.449	3.374 \pm 1.533	0.007	0.043
CDCA	1.010 \pm 0.437	1.246 \pm 0.739	0.984	0.705 \pm 0.293	2.539 \pm 1.699	0.003	0.008
Secondary Bile Acids							
DCA	4.018 \pm 0.918	6.464 \pm 2.303	0.479	3.974 \pm 0.885	7.797 \pm 2.853	0.979	0.951
iso-DCA	0.372 \pm 0.065	0.507 \pm 0.131	0.898	0.360 \pm 0.052	0.482 \pm 0.095	0.985	0.940
LCA	4.694 \pm 0.880	4.909 \pm 1.115	0.574	3.842 \pm 0.913	5.034 \pm 1.105	0.247	0.202
iso-LCA	1.146 \pm 0.229	0.895 \pm 0.148	0.119	1.003 \pm 0.170	1.103 \pm 0.147	0.115	0.230
HDCA	0.344 \pm 0.113	0.406 \pm 0.082	0.697	0.383 \pm 0.141	0.362 \pm 0.041	0.759	0.500
UDCA	0.338 \pm 0.102	0.516 \pm 0.193	0.994	0.341 \pm 0.082	0.450 \pm 0.108	0.208	0.528
Total Fecal Bile Acids	13.572 \pm 1.861	17.233 \pm 3.811	0.538	12.409 \pm 2.010	21.141 \pm 5.440	0.559	0.789
CDCA%	6.89 \pm 2.32	5.97 \pm 1.92	0.821	5.56 \pm 1.77	6.50 \pm 2.75	0.002	0.018

¹Values are mean \pm SEM, $n=16$ for prebiotic and $n=13$ for placebo. CA- cholic acid, CDCA- chenodeoxycholic acid, DCA- deoxycholic acid, LCA- lithocholic acid, HDCA- hyodeoxycholic acid, UDCA- ursodeoxycholic acid, CDCA% - proportion of CDCA to total fecal bile acids.

Table 4. Within group changes of microbial abundance assessed using Illumina 16S rRNA gene tag sequencing in children (7-12 years) with overweight and obesity consuming oligofructose-enriched inulin

(prebiotic) or placebo for 16 weeks¹

Taxonomic Group	Mean % bacterial abundance \pm SD					
	Prebiotics			Placebo		
	Initial	Final	<i>P</i> value (adj. val.)	Initial	Final	<i>P</i> value (adj. val.)
Phyla						
Actinobacteria	8.6 \pm 4.7	13.5 \pm 8.6	0.008 (0.217)	9.4 \pm 5.5	9.5 \pm 6.4	0.410 (0.938)
Bacteroidetes	14.7 \pm 8.6	16.7 \pm 14.8	0.663 (0.913)	14.7 \pm 9.5	19.1 \pm 14.2	0.244 (0.900)
Firmicutes	68.6 \pm 8.5	62.8 \pm 13.5	0.338 (0.842)	68.0 \pm 7.9	63.8 \pm 13.2	0.201 (0.881)
Proteobacteria	4.8 \pm 2.4	4.0 \pm 2.1	0.151 (0.704)	4.5 \pm 2.5	4.2 \pm 2.2	0.379 (0.933)
Verrucomicrobia	0.6 \pm 0.8	0.6 \pm 0.9	0.887 (0.933)	1.1 \pm 2.7	1.7 \pm 3.4	0.754 (0.965)
Genera						
<i>Actinomyces</i>	0.017 \pm 0.016	0.016 \pm 0.017	0.408 (0.866)	0.020 \pm 0.016	0.009 \pm 0.008	0.018 (0.487)
<i>Bifidobacterium</i>	5.821 \pm 3.719	9.843 \pm 6.242	0.012 (0.217)	5.797 \pm 4.280	6.655 \pm 6.168	0.349 (0.928)
<i>Clostridium XVIII</i>	0.611 \pm 0.534	0.637 \pm 0.684	0.728 (0.920)	0.916 \pm 0.578	0.581 \pm 0.390	0.005 (0.487)
<i>Collinsella</i>	2.072 \pm 1.706	2.966 \pm 2.947	0.045 (0.419)	2.915 \pm 2.110	2.410 \pm 2.126	0.485 (0.947)
<i>Dorea</i>	2.874 \pm 1.226	2.305 \pm 1.251	0.061 (0.491)	2.988 \pm 1.147	2.418 \pm 2.241	0.016 (0.487)
<i>Eggerthella</i>	0.074 \pm 0.125	0.096 \pm 0.150	0.384 (0.858)	0.084 \pm 0.109	0.061 \pm 0.107	0.033 (0.550)
<i>Ruminococcus</i>	2.346 \pm 1.665	1.457 \pm 1.592	0.026 (0.311)	1.761 \pm 1.361	1.594 \pm 1.107	0.842 (0.969)
OTUs						
OTU_1157 (<i>Anaerostipes</i> sp., 97%)	0.034 \pm 0.025	0.059 \pm 0.057	0.037 (0.331)	0.050 \pm 0.043	0.048 \pm 0.048	0.755 (0.931)
OTU_2013 (<i>Anaerostipes butyraticus</i> , 98%)	0.141 \pm 0.101	0.244 \pm 0.231	0.045 (0.357)	0.248 \pm 0.312	0.223 \pm 0.317	0.349 (0.861)
OTU_2492 (<i>Bacteriodes vulgatus</i> , 100%)	0.982 \pm 0.967	0.459 \pm 0.522	0.005 (0.155)	0.650 \pm 0.909	0.339 \pm 0.456	0.258 (0.821)
OTU_1554 (<i>bacterium mpn-isolate</i> , 98%)	0.031 \pm 0.030	0.014 \pm 0.017	0.016 (0.239)	0.023 \pm 0.033	0.012 \pm 0.016	0.414 (0.881)
OTU_2169 (<i>Bifidobacterium adolescentis</i> , 99%)	0.004 \pm 0.004	0.008 \pm 0.009	0.003 (0.154)	0.004 \pm 0.004	0.007 \pm 0.009	0.230 (0.804)
OTU_30 (<i>Bifidobacterium bifidum</i> , 100%)	0.596 \pm 0.750	0.972 \pm 1.275	0.028 (0.300)	0.285 \pm 0.496	0.330 \pm 0.497	0.563 (0.909)
OTU_2403 (<i>Bifidobacterium longum</i> , 98%)	0.001 \pm 0.001	0.005 \pm 0.006	0.005 (0.155)	0.002 \pm 0.003	0.002 \pm 0.004	0.813 (0.935)
OTU_1682 (<i>Clostridiales bacterium</i> , 98%)	0.009 \pm 0.008	0.018 \pm 0.022	0.026 (0.294)	0.017 \pm 0.026	0.018 \pm 0.026	0.629 (0.918)
OTU_2559 (<i>Clostridium</i>	2.986 \pm	1.998 \pm	0.024	2.409 \pm	2.517 \pm	0.712

<i>clostridioforme</i> , 99%)	1.455	1.347	(0.281)	0.988	1.140	(0.927)
OTU_160 (<i>Clostridium</i> <i>scindens</i> , 100%)	0.024 ± 0.043	0.050 ± 0.099	0.036 (0.327)	0.022 ± 0.045	0.016 ± 0.038	0.148 (0.726)
OTU_9 (<i>Collinsella</i> <i>aerofaciens</i> , 100%)	1.810 ± 1.518	2.658 ± 2.693	0.021 (0.267)	2.643 ± 1.907	2.184 ± 1.923	0.442 (0.887)
OTU_59 (<i>Eubacterium</i> <i>eligens</i> , 100%)	0.230 ± 0.326	0.118 ± 0.180	0.006 (0.156)	0.288 ± 0.425	0.316 ± 0.568	0.478 (0.895)
OTU_2516 (<i>Faecalibacterium</i> <i>prausnitzii</i> , 98%)	2.010 ± 1.634	0.947 ± 0.673	0.002 (0.153)	1.173 ± 0.793	1.670 ± 1.470	0.182 (0.764)
OTU_32 (<i>Roseburia</i> sp., 100%)	0.689 ± 0.686	0.457 ± 0.413	0.029 (0.306)	0.491 ± 0.406	0.424 ± 0.332	0.551 (0.907)
OTU_2376 (<i>Ruminococcus</i> <i>gnavus</i> , 97%)	0.004 ± 0.004	0.002 ± 0.002	0.009 (0.185)	0.004 ± 0.006	0.004 ± 0.006	0.572 (0.911)
Alpha Diversity						
Shannon Index	6.22 ± 0.40	5.93 ± 0.45	0.005	6.11 ± 0.42	5.89 ± 0.39	0.008
Simpson Index	0.97 ± 0.01	0.96 ± 0.02	0.001	0.97 ± 0.01	0.96 ± 0.01	0.003
Observed OTUs	460.4 ± 68.0	439.6 ± 70.1	0.107	435.7 ± 58.8	420.3 ± 54.1	0.132

¹*n*=20 for prebiotic and *n*=16 for placebo. *P* value (adj. val.), FDR significance set at 0.2.



