# Effect of Prebiotic on Microbiota, Intestinal Permeability, and Glycemic Control in Children With Type 1 Diabetes

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**Context:** Patients with type 1 diabetes (T1D) have lower microbiota diversity and distinct gut microbial profiles that have been linked to changes in intestinal permeability. Prebiotics are nondigestible carbohydrates that alter gut microbiota and could potentially improve glycemic control and reduce intestinal permeability and thereby insulin sensitivity.

**Objective:** To determine the effect of prebiotics on glycemic control, gut microbiota, and intestinal permeability in children with T1D.

**Design:** A randomized, placebo-controlled trial in children 8 to 17 years of age with T1D using placebo or prebiotic oligofructose-enriched inulin for 12 weeks. Baseline, 3-month, and 6-month assessments included HbA1c, C-peptide, gut microbiota, intestinal permeability, frequency of diabetic ketoacidosis (DKA), and severe hypoglycemia.

**Results:** Forty-three subjects were randomized and 38 completed the study. The groups were similar at baseline: prebiotic (N = 17), age 12.5 years (SD of 2.8), HbA1c 8.02% (SD of 0.82); placebo (N = 21), age 12.0 years (SD of 2.6), HbA1c 8.08% (SD of 0.91). No significant differences were found in the frequency of DKA or severe hypoglycemia. At 3-months, C-peptide was significantly higher ($P = 0.029$) in the group who received prebiotics, which was accompanied by a modest improvement in intestinal permeability ($P = 0.076$). There was a significant increase in the relative abundance of *Bifidobacterium* within the prebiotic group at 3 months that was no longer present after the 3-month washout. The placebo group had significantly higher relative abundance of *Streptococcus*, *Roseburia inulinivorans*, *Terrisporobacter*, and *Faecalitalea* compared with the prebiotic group at 3 months.

**Conclusion:** Prebiotics are a potentially novel, inexpensive, low-risk treatment addition for T1D that may improve glycemic control. Further larger-scale trials are needed. (***J Clin Endocrinol Metab* 104: 4427–4440, 2019**)

The gut microbiota plays a key role in health and it is increasingly being recognized as a contributor to various disease states when an imbalance occurs. Both animal (1) and human studies have reported a difference in microbial composition between those that develop diabetes from those that did not develop diabetes (2). In

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Abbreviations: A1C, HbA1c; BB, BioBreeding; DKA, diabetic ketoacidosis; GLP, glucagon-like peptide; Lac/Man, lactulose/mannitol; LEfSe, linear discriminant analysis effect size; LPS, lipopolysaccharide; NOD, nonobese diabetic; SCFA, short-chain fatty acid; T1D, type 1 diabetes.

humans, gut microbiota composition differs between healthy controls, those with β-cell autoantibody [*i.e.* at risk for type 1 diabetes (T1D)] (3), and patients with established T1D (4, 5). This altered microbiota is termed dysbiosis, and in comparison with the microbiota of healthy controls, it has a lower abundance of bifidobacteria and a higher abundance of Gram-negative bacteria (3).

Dysbiosis contributes to metabolic dysregulation. Animal studies have shown that a dysbiotic gut microbiota generates high levels of lipopolysaccharides (LPSs), which leads to inflammation of the intestinal mucosa, loss of tight junction integrity between epithelial cells, and increased intestinal permeability (6, 7). This may contribute to deterioration of glycemic control because LPS will cross the leaky intestinal epithelium into the circulation, resulting in high serum LPS levels or endotoxemia, causing systemic inflammation, insulin resistance, and poor glycemic control (8).

Studies have shown that in comparison with healthy individuals, those with β-cell autoimmunity or established T1D have impaired intestinal epithelial barrier function (9–12) with increased intestinal permeability, or a "leaky gut" (9). The leaky gut has also been shown to predate onset of T1D in humans (13), potentially playing a role in pathogenesis of the disease (14–16).

In animal studies, interventions that change the gut microbiota and correct dysbiosis have been shown to alter intestinal permeability and alter the disease course in T1D (17). In diabetes-prone BioBreeding (BB) rats and nonobese diabetic (NOD) mice, manipulation of gut microbiota by antibiotic treatment or fecal transfer leads to altered microbiota composition and changes diabetes incidence (1, 18). One potentially effective intervention in this context is a prebiotic fiber, such as inulin or oligofructose. Prebiotics are substrates that are selectively used by host microorganisms and confer a health benefit to the host (19). Prebiotic fiber has been shown to increase the abundance of *Bifidobacterium*, which in turn produce short-chain fatty acids (SCFAs) (19). SCFAs bind to G-protein–coupled receptors on intestinal L-cells to stimulate the release of glucagon-like peptide and peptide YY, resulting in increased insulin and decreased glucagon release, both of which lower blood glucose (20). SCFAs have also been shown to dampen inflammation via a decrease in intestinal permeability and reduction in circulating endotoxins (20) and systemic inflammation. Indeed, prebiotics improved HbA1c (A1C), postprandial glycemic excursion, and inflammatory markers in patients with type 2 diabetes (21, 22). As such, prebiotics are a potentially novel, inexpensive, low-risk treatment addition for diabetes that may improve glycemic control by changes in gut microbiota, gut permeability, and inflammation.

The aim of this study was to assess the effect of using a prebiotic to alter gut microbiota and intestinal permeability in children with T1D and assess whether such changes could improve glycemic control. The main objective of this study was to determine the effect of a 12-week dietary intervention with the prebiotic oligofructose-enriched inulin compared with placebo on glycemic control as measured by A1C in children diagnosed with T1D for at least 1 year. The secondary objective was to examine the differences in gut microbiota and intestinal permeability and determine whether these factors correlate with changes seen in A1C and C-peptide.

## Materials and Methods

### Study design

The protocol for this study has been previously described (23). The study was a single-center, randomized, double-blind, placebo-controlled pilot study of prebiotic treatment (oligofructose-enriched inulin) on gut microbiota, intestinal permeability, and glycemic control in children aged 8 to 17 years that had T1D for at least 1 year.

### Ethics

Ethics approval was obtained from the Conjoint Health Research Ethics Board at the University of Calgary on 10 June 2015 (REB15-0695).

### Trial registration

The trial was registered at ClinicalTrials.gov on 10 March 2015 (NCT02442544).

### Study population

Patients were recruited from the diabetes clinic at a tertiary care center (Alberta Children's Hospital, Calgary, AB, Canada) from February 2016 to March 2017. Patients were included when they were aged 8 to 17 years, diagnosed with T1D for at least 12 months, and received follow-up care at the Alberta Children's Hospital diabetes clinic.

Patients were excluded when they had an A1C >10% in the previous 6 months, had a chronic medical condition that could affect gut microbiota (*e.g.*, Crohn disease, cystic fibrosis, irritable bowel syndrome), were receiving medications or supplements that could affect gut microbiota (*e.g.*, antibiotics, probiotics, prebiotics, laxatives), or had a positive celiac disease screen.

### Recruitment

Patients were given a pamphlet about the study during their regular diabetes clinic visits and referred to the research team when they indicated an interest in the study. Additionally, the study was advertised in clinic newsletters.

### Consent

Written, informed consent from legal guardians was obtained for all participants. Assent was obtained from children aged 8 to 14 years and consent from those ≥14 years of age.

## Randomization

Participants were randomized 1:1 to the prebiotic or placebo group using computer-generated random numbers. Both the patients and the research team were blinded to the group assignments.

## Intervention

The placebo group received 3.3 g of maltodextrin orally every day (Agenamalt 20.222; Agrana Starch, Konstanz, Germany). The prebiotic group received 8 g of oligofructose-enriched inulin orally every day (chicory root–derived Synergy1; Beneo, Mannheim, Germany). Inulin and oligofructose are approved as food ingredients in Canada and have been used previously in clinical trials (24, 25). The dose of prebiotic (8 g/d) was chosen because it has been used previously in randomized controlled trials in children and youth (26–28). In healthy pubertal adolescents, 8 g/d oligofructose-enriched inulin resulted in a significantly smaller increase in fat mass during 1 year compared with controls (28). In children who were overweight or with obesity, 8 g/d oligofructose-enriched inulin was shown to decrease body weight *z* score and percentage body fat as well as improve appetite control compared with placebo with minimal adverse effects (26, 27).

The placebo maltodextrin was provided at an isocaloric dose to the prebiotic and is an appropriate placebo given its similar taste and physical appearance to the prebiotic (26, 27). The low dose provided would minimize any potential effects from the maltodextrin itself. In the placebo group, the mean weight of the participants was 47.3 kg (SD of 3.3). They were given 3.3 g/d maltodextrin, which is ~0.07 g/kg/d. This very low dose is in contrast to a study in mice (29) that showed some adverse effects of maltodextrin on intestinal inflammation when a 135-fold higher dose of 9.5 g/kg/d was provided.

Both the prebiotic and placebo were provided to participants in powder form in identical foil preweighed packets. Instructions were to mix the contents of the packet with 250 mL of water until dissolved and to drink it 15 to 20 minutes prior to the evening meal. For the first 2 weeks, participants were asked to take half of the dose to minimize gastrointestinal side effects. The full dose was taken for the remaining 10 weeks, for a total of 3 months of intervention followed by a 3-month washout period with no intervention.

Participants were asked to record any diabetes-related or gastrointestinal adverse reactions [*e.g.*, frequency of mild hypoglycemia (symptoms of hypoglycemia with a blood glucose <4 mmol/L and able to self-treat with oral rapid-acting carbohydrate), severe hypoglycemia (symptoms of hypoglycemia with a blood glucose <4 mmol/L but that required assistance with treatment due to decreased level of consciousness or a seizure), diabetic ketoacidosis (DKA), abdominal pain, bloating, discomfort, flatulence]. At the end of the 12 weeks, participants were asked to return any remaining packets of placebo or prebiotic to assess for compliance.

## Data collection

Demographic information was collected at baseline. Anthropometric measures and assessment of insulin regimens, frequency of DKA in the previous 3 months, frequency of severe hypoglycemia in the previous 3 months, and average number of mild hypoglycemia per week in the preceding 3 months were assessed at baseline, 3 months, and at 6 months (after a 3-month washout period with no intervention).

### *Glycemic control, inflammatory markers, glucagon-like peptide-1, and glucagon-like peptide-2*

Baseline, 3-month, and 6-month blood samples were drawn for serum C-peptide, A1C, serum inflammatory markers (IL-6, IFN-$\gamma$, TNF-$\alpha$, and IL-10), glucagon-like peptide (GLP)-1, and GLP-2. A1C was measured by a turbidimetric inhibition immunoassay (Integra 800 CTS; Roche, Basel, Switzerland), and serum C-peptide was measured by a chemiluminescent assay (Immulite 2000; Siemens, Erlangen, Germany). Serum inflammatory markers were analyzed using Milliplex human cytokine magnetic bead panel (Multiplex) kits (Millipore, St. Charles, MO) at Eve Technologies (Calgary, AB, Canada). GLP-1 and GLP-2 were measured using ELISA kits (Millipore).

### *Intestinal permeability*

Intestinal permeability was assessed at baseline, 3 months, and 6 months. Participants were asked to consume a regular evening meal and then 3 hours later, prior to bedtime, drink a solution containing lactulose (5 g) and mannitol (2 g) in 200 mL of water (BioSource International, Montreal, QC, Canada). Urine for the following 12 hours was collected with 5 mL of thymol in the storage container for preservation, and stored frozen. HPLC was used to analyze urine for the lactulose and mannitol content. The fraction of the ingested dose recovered, that is, the lactulose/mannitol (Lac/Man) ratio, in the urine sample was calculated and compared between the two groups (12, 30).

### *Gut microbiota profiling*

Stool samples were collected at baseline, 3 months, and 6 months as previously described (31, 32). Participants collected one tablespoon of stool and stored it in the home freezer (−20°C) until it was delivered on ice to the research laboratory and stored at −80°C until analyzed. Bacterial DNA was extracted from stool using a FastDNA SPIN kit for feces (MP Biomedicals, Lachine, QC, Canada) followed by ethanol precipitation purification. DNA was quantified using a Qubit double-stranded DNA assay (Promega, Madison, WI) and diluted to 5 ng/μL concentration. Bacterial community composition was determined as per our previously published protocol (33) following Illumina's 16S rRNA amplicon sequencing protocol on the MiSeq platform (Illumina, San Diego, CA). The 16S hypervariable regions V3 to V4 were sequenced at the Centre for Health Genomics and Informatics (University of Calgary). Sequence analysis was performed in R (version 3.5.2). Filtering of raw sequence reads for quality was performed using the R package dada2 (version 1.10.1). A table of amplicon sequence variants was generated using dada2 and taxonomic classifications assigned using the Silva 132 database as a reference. $\beta$-Diversity was estimated by nonmetric multidimensional scaling using the function metaMDS in the R package vegan (version 2.5.2) with a Bray–Curtis distance matrix (stress, 0.177). $\alpha$-Diversity was measured by calculating the Shannon index, Simpson index, and Chao1 metrics. Differential abundance analysis between groups was carried out using the linear discriminant analysis effect size (LEfSe) algorithm (34), with $\alpha = 0.05$, to determine significance of differentially abundant features.

Given that 16S rRNA sequencing generates relative abundance data, we also quantified the absolute abundance of *Bifidobacterium* spp. with quantitative PCR according to our

previous work (31, 32) using the Bio-Rad iCycler (Bio-Rad Laboratories, Mississauga, ON, Canada).

### Sample size

For this study, the sample size was calculated for a two-sided *t* test comparing two independent samples. Based on previous follow-up data from the Alberta Children's Hospital Diabetes Clinic, the mean A1C at baseline in both groups was estimated to be 8.4% with an SD of 1.3 for each group. A clinically significant change in absolute A1C of 1.5 was used. For a power of 80% and $\alpha$ of 0.05, the number of subjects needed per arm of the study was 12. If a dropout rate of 20% was assumed, then ~15 subjects per arm of the study were required for this initial study.

### Statistical analysis

Statistical analysis was performed using SPSS 22.0 software (IBM, New York, NY). Results were considered statistically significant at $P \leq 0.05$. Baseline descriptive data between the control and intervention group were compared using $\chi^2$ for categorical variables and *t* tests for continuous variables. The primary outcome of A1C was expressed as mean A1C values with SDs. A two-sided *t* test was used to compare A1C between the placebo and prebiotic group, and differences induced by the 3-month intervention in C-peptide, inflammatory markers (IL-6, IFN-$\gamma$, TNF-$\alpha$, and IL-10), GLP-1 and GLP-2, and intestinal permeability). Spearman correlation analysis was used to assess the relationship between changes in gut microbial abundance and other markers.

## Results

### Participant characteristics

A total of 470 patients were approached for the study, 46 consented to participate, 43 were randomized, 5 withdrew, and 38 completed the study (Fig. 1). At baseline, both groups were of similar age and had similar glycemic control (Table 1). All patients had diabetes for at least 1 year (range, 1 to 13 years). In terms of compliance with intervention, 4 out of 17 patients in the prebiotics group and 5 out of 20 patients in the placebo group took <80% of the packets.

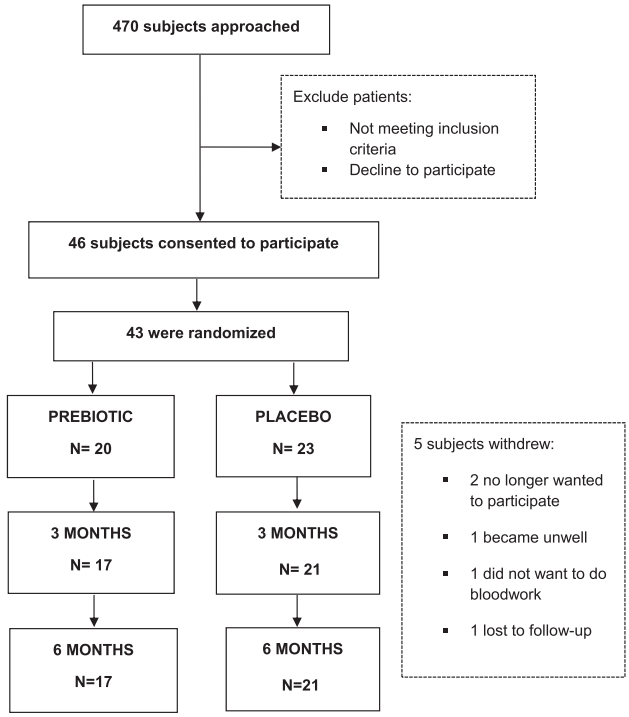


**Figure 1.** Study flow diagram.

### Metabolic changes

Because prebiotics can correct dysbiosis with the potential of reducing systemic inflammation and insulin resistance, we determined A1C in both groups before and after treatment, as well as at 3 months after the end of treatment to detect any "washout" effect. There was no

**Table 1. Baseline Demographics**

| Baseline Demographics | Prebiotic (N = 17) | Placebo (N = 21) | *P* Value |
|---|---|---|---|
| Male/female | 12/5 | 7/14 | 0.022 |
| Age, y | 12.52 (2.76) | 11.94 (2.61) | 0.561 |
| Insulin dose, U/kg/d | 0.87 (0.25) | 0.92 (0.29) | 0.928 |
| Age at T1D diagnosis, y | 5.18 (3.37) | 7.27 (4.37) | 0.115 |
| Duration of T1D, y | 7.31 (3.93) | 4.70 (3.07) | 0.027 |
| A1C, % | 8.02 (0.82) | 8.08 (0.91) | 0.854 |
| C-peptide, pg/mL | 262.96 (751.02) | 208.79 (303.10) | 0.764 |
| Lac/Man ratio | 0.029 (0.017) | 0.025 (0.008) | 0.344 |
| GLP-1, pg/mL | 248.13 (940.46) | 16.27 (23.04) | 0.264 |
| GLP-2, ng/mL | 1.56 (0.72) | 1.69 (1.06) | 0.681 |
| GIP, pg/mL | 94.76 (97.67) | 76.67 (83.49) | 0.542 |
| IL-6, pg/mL | 43.97 (148.59) | 7.35 (8.95) | 0.266 |
| IL-10, pg/mL | 15.10 (36.17) | 12.98 (17.91) | 0.816 |
| IFN-$\gamma$, pg/mL | 444.13 (1792.58) | 20.57 (44.70) | 0.285 |
| TNF-$\alpha$, pg/mL | 16.08 (52.33) | 3.89 (1.75) | 0.292 |

Results are presented as mean (SD).

Abbreviation: GIP, gastric inhibitory polypeptide.

significant change in A1C from baseline to 3 months in either the placebo or the prebiotic group (Table 2). We also observed no difference in the change in inflammatory markers, such as IL-6, IL-10, TNF-$\alpha$, and IFN-$\gamma$, between the two groups (Table 2). Owing to the known effect of prebiotic-associated bacterial metabolites (*i.e.*, SCFAs) on stimulation of glucagon-like peptides, we measured serum levels of GLP-1 and GLP-2 before and after the prebiotic treatment. In this study, we observed no significant difference in GLP-1 and GLP-2 in response to the treatment between the placebo and prebiotic group (Table 2).

Next, we examined the treatment effect on C-peptide because there is a potential link between dysbiosis and diabetes progression. We sought to determine whether prebiotic treatment modified dysbiosis and residual $\beta$-cell function. Interestingly, although most patients had minimal C-peptide levels, the group that received prebiotic treatment had significant preservation of their C-peptide levels during the 3-months intervention whereas the placebo group showed a drop in C-peptide during the same time period (Table 2).

### Intestinal permeability

Previous work suggested that T1D is associated with increased intestinal permeability, which may contribute to disease progression, systemic inflammation, and poor metabolic control (35). In this study, we determined intestinal permeability by measuring the ratio of lactulose to mannitol recovered in the urine after oral ingestion of the two sugars. We found that one third of the T1D patients had a Lac/Man ratio >0.03 at baseline, a level that is significantly higher than that in healthy controls and is considered abnormal (36). After 3 months of oligofructose-enriched inulin treatment, the prebiotic group had a decrease in intestinal permeability whereas the placebo group had an increase in their intestinal permeability, although this difference did not reach statistical significance ($P = 0.076$) (Fig. 2; Table 2).

**Table 2. Changes in Metabolic Parameters and Intestinal Permeability From Baseline in Children With T1D Mellitus Consuming Oligofructose-Enriched Inulin (Prebiotic) or Placebo for 3 mo**

| Change From Baseline | Prebiotic | Placebo | *P* Value |
|---|---|---|---|
| | N = 17 | N = 21 | |
| A1C, %, at 3 mo | −0.08 (0.50) | +0.06 (0.91) | 0.592 |
| A1C, %, at 6 mo | +0.02 (0.58) | +0.22 (0.96) | 0.456 |
| | N = 16 | N = 19 | |
| C-peptide, pg/mL, at 3 mo | +43.89 (93.02) | −56.43 (154.42) | 0.029 |
| C-peptide, pg/mL, at 6 mo | +4.26 (31.54) | −26.64 (92.1) | 0.210 |
| | N = 12 | N = 12 | |
| Lac/Man at 3 mo | −0.005 (0.005) | +0.011 (0.021) | 0.076 |
| Lac/Man at 6 mo | −0.009 (0.006) | +0.008 (0.014) | 0.347 |
| | N = 15 | N = 19 | |
| GLP-1 at 3 mo | 4.06 (12.47) | 0.96 (14.09) | 0.524 |
| GLP-1 at 6 mo | −1.85 (6.38) | −5.37 (16.56) | 0.443 |
| | N = 16 | N = 20 | |
| GLP-2 at 3 mo | 0.07 (1.00) | 0.09 (1.33) | 0.956 |
| GLP-2 at 6 mo | 0.06 (1.24) | −0.29 (0.77) | 0.305 |
| | N = 16 | N = 20 | |
| GIP at 3 mo | 2.49 (121.35) | 22.43 (162.40) | 0.684 |
| GIP at 6 mo | −27.79 (146.80) | −21.46 (119.82) | 0.887 |
| | N = 15 | N = 21 | |
| IL-6 at 3 mo | 8.94 (25.41) | 0.84 (9.01) | 0.185 |
| IL-6 at 6 mo | 0.27 (4.88) | −1.65 (5.88) | 0.309 |
| | N = 16 | N = 20 | |
| IL-10 at 3 mo | 2.88 (10.88) | −5.98 (18.16) | 0.093 |
| IL-10 at 6 mo | 11.23 (37.10) | −5.68 (19.02) | 0.085 |
| | N = 15 | N = 19 | |
| IFN-$\gamma$ at 3 mo | −0.72 (14.59) | −8.85 (46.92) | 0.523 |
| IFN-$\gamma$ at 6 mo | 1.51 (12.57) | −9.28 (45.95) | 0.385 |
| | N = 16 | N = 20 | |
| TNF-$\alpha$ at 3 mo | 1.59 (3.95) | −0.24 (1.52) | 0.058 |
| TNF-$\alpha$ at 6 mo | 0.26 (1.18) | −0.46 (1.33) | 0.101 |

Results are presented as mean (SD). Results represent the measurement at 3 or 6 mo after the start of intervention minus that at baseline. Intervention (prebiotics or placebo) was provided between baseline and 3 mo, followed by a 3-mo washout (6-mo measures). Not all subjects had complete data available for analysis (*e.g.*, sample insufficient, not collected), and therefore the N is listed for each variable tested). The change in each parameter was compared between the probiotic and the placebo group by a Student *t* test. Statistical significance was set at $P < 0.05$.

Abbreviation: GIP, gastric inhibitory polypeptide.

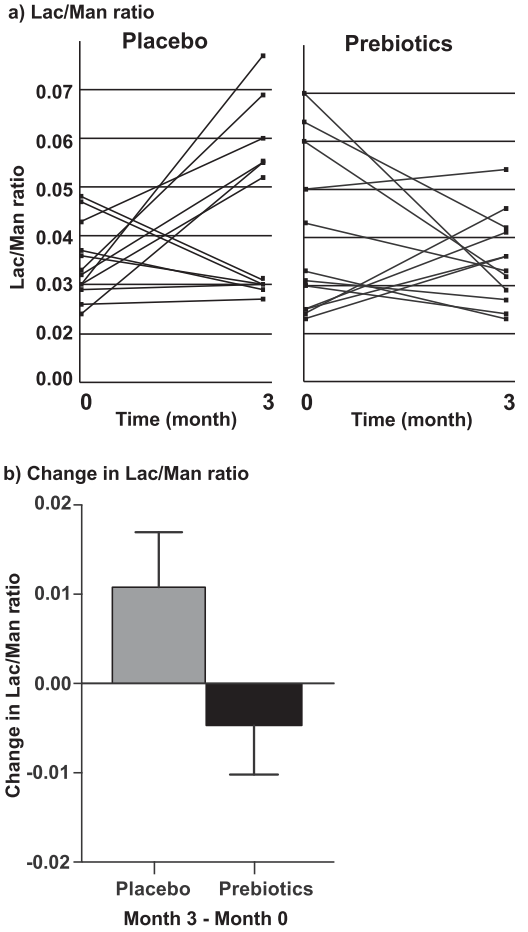


**Figure 2.** Intestinal permeability as measured by the Lac/Man ratio recovered from the urine after oral administration. (a) Lac/Man ratio in patients at baseline and at 3 mo after intervention. Each line represents one patient. N = 12 for each group (urine sample available for analysis). (b) Change in the Lac/Man ratio in the placebo (+0.011) and prebiotic (−0.005) group ($P = 0.076$).

## Gut microbiota

$\alpha$-Diversity (within-sample diversity) was slightly but significantly decreased in the prebiotic group ($P = 0.035$) according to the Shannon index but not Simpson index or observed operational taxonomic units (Table 3). For $\beta$-diversity (overall microbial community structure), a permutational multivariate ANOVA showed that there was a significant effect of treatment on the distribution of the samples ($R^2 = 0.02109$; $P = 0.003$). A test of homogeneity of group dispersions was not significant, meaning the significance of the permutational multivariate ANOVA was due to differences in the centroid of the groups and not due to differences in group dispersions (Fig. 3). Bar plots of relative abundance at the phylum and family level are shown in Fig. 4. An analysis of differentially abundant features between groups showed that the relative abundance of *Bifidobacterium* and specifically *Bifidobacterium longum* was significantly increased at 3 months in the prebiotic group (Fig. 5). In addition to *Bifidobacterium*, the observed increase in Actinobacteria with prebiotic vs placebo was also driven by significantly higher relative abundance of Coriobacteriales. At 3 months, the placebo group had significantly higher relative abundance of *Streptococcus*, *Roseburia inulinivorans*, *Terrisporobacter*, and *Faecalitalea,* among other taxa compared with prebiotic. After the 3-month washout (6-month time point), most of the above-noted differences were no longer present; however, the prebiotic group continued to show increased relative abundance of Coriobacteriales over placebo (Fig. 6). The placebo group had increased relative abundance of Bacteroidales and Proteobacteria at 6 months compared with the prebiotic group.

Given the known bifidogenic effects of prebiotic (19), we quantified *Bifidobacterium* spp. using quantitative PCR (relative abundance of *Bifidobacterium* spp. per total bacteria) and showed a significant time-by-diet interaction ($P = 0.007$). At 3 months, the relative abundance of *Bifidobacterium* spp. was significantly higher (3.34% ± 0.58%) than placebo (1.71% ± 0.34%). By 6 months, no differences were present between the prebiotic (1.62% ± 0.27%) and placebo (1.94% ± 0.46%).

## Correlations

At baseline, there was a significant correlation between IL-10 and the Lac/Man ratio ($r_s = -0.487$, $P < 0.05$) and the relative abundance of *Terrisporobacter* ($r_s = -0.525$, $P < 0.05$) in the prebiotic group (Table 4). At the end of the prebiotic intervention (3 mo), there was a significant correlation between A1C and the Lac/Man ratio ($r_s = 0.628$, $P < 0.01$) and a negative correlation between *Terrisporobacter* and C-peptide ($r_s = -0.484$, $P < 0.05$). After the washout (6 months), there was a significant correlation between A1C and C-peptide ($r_s = -0.668$, $P < 0.01$) in the prebiotic group. In the placebo group (Table 5), there was a significant correlation between C-peptide and the relative abundance of *Terrrisporobacter* ($r_s = -0.459$, $P < 0.05$). At 3 months, A1C was significantly correlated with TNF$\alpha$ ($r_s = 0.440$, $P < 0.05$), C-peptide ($r_s = -0.466$, $P < 0.05$), and the Lac/Man ratio ($r_s = 0.465$, $P < 0.05$). After the washout (6 months), the correlation between A1C and C-peptide ($r_s = -0.567$, $P < 0.01$) and the Lac/Man ratio ($r_s = 0.519$, $P < 0.05$) remained significant.

## Side effects

Gastrointestinal side effects were reported by the same number of participants in each group at 3 months (one in each group) and 6 months (two in each group). These included abdominal pain, cramping, soft stool, and bloating. There were no significant differences in the

**Table 3. α-Diversity Metrics Derived From Illumina 16S rRNA Sequencing in Children With T1D Mellitus Consuming Oligofructose-Enriched Inulin (Prebiotic) or Placebo for 3 mo**

| | Prebiotics (N = 17) | | | Placebo (N = 21) | | | |
|---|---|---|---|---|---|---|---|
| α-Diversity | Initial | Final | Within-Group *P* Value | Initial | Final | Within-Group *P* Value | Between- Group *P* Value |
| Shannon index | 5.30 ± 0.08 | 5.13 ± 0.08 | 0.035 | 5.27 ± 0.07 | 5.23 ± 0.09 | 0.551 | 0.115 |
| Simpson index | 0.956 ± 0.002 | 0.951 ± 0.002 | 0.104 | 0.955 ± 0.002 | 0.954 ± 0.003 | 0.832 | 0.238 |
| Observed OTUs | 201.3 ± 10.2 | 193.4 ± 11.6 | 0.173 | 188.5 ± 11.3 | 192.1 ± 13.1 | 0.523 | 0.103 |

Abbreviation: OTU, operational taxonomic unit.

self-reported adverse events during the study period (Table 6). The average insulin dose (U/kg/d) was 0.92 (0.28) in the prebiotic group and 0.91 (0.22) in the placebo group at the 3-months follow-up and 0.91 (0.24) in the prebiotic group and 0.90 (0.27) in the placebo group at the 6-month follow-up.

## Discussion

The management of T1D in children is currently based on intensive multiple daily subcutaneous insulin injections or infusion and frequent glucose monitoring and it remains challenging to obtain optimal glycemic control (37). An adjunctive oral supplement, such as the prebiotic used in this study, to improve glycemic control could prove beneficial in this population. In the current study, there was no difference in the number of adverse events such as DKA, severe hypoglycemia, and nonsevere hypoglycemia per week between the prebiotic and placebo group. Additionally, few participants reported any gastrointestinal side effects, which supports that this is a well-tolerated intervention.

Kellow *et al.* (21) published a systematic review on the metabolic benefits of prebiotics in human randomized controlled trials. Meta-analysis indicated a statistically significant effect of prebiotics on reduction in postprandial glucose levels. No pediatric studies or populations with T1D were identified for inclusion in the systematic review. In the current study, we saw an increase in C-peptide at 3 months in the prebiotic group compared with the placebo group ($P = 0.029$), which suggests improved $\beta$-cell function, which clinically could lead to improved glycemic control. Although our primary outcome of A1C did not show a significant decrease with

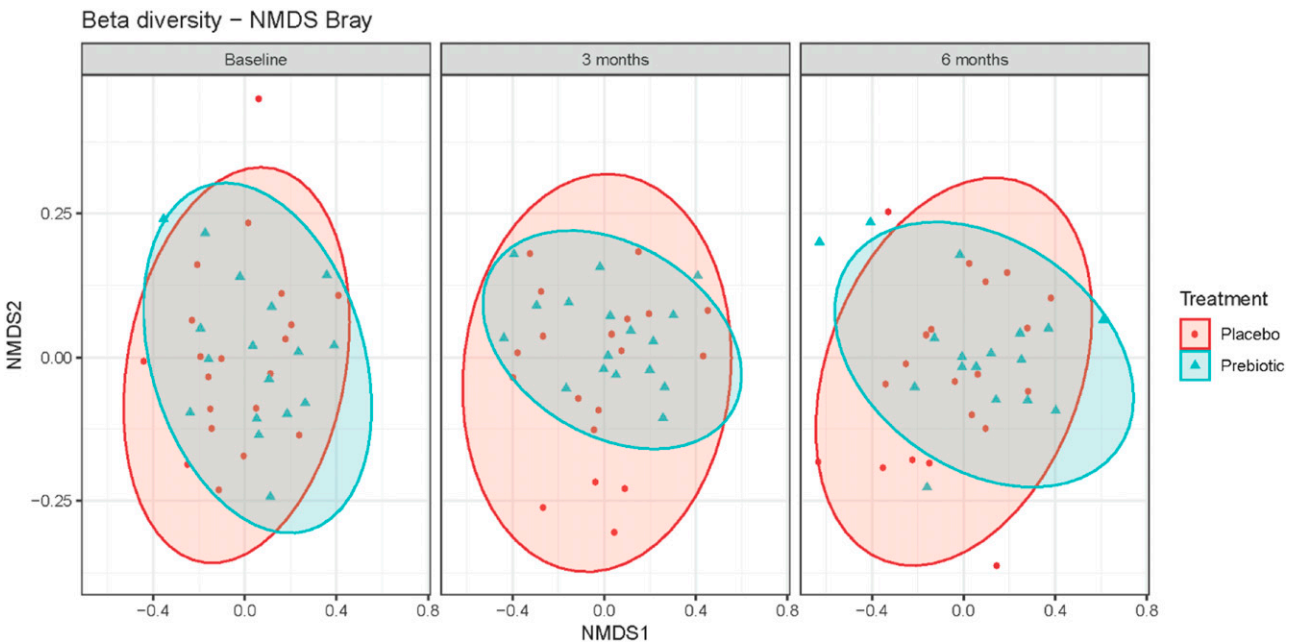


**Figure 3.** Bacterial community clustering in fecal samples derived from children with T1D before and after consuming placebo or prebiotic for 3 mo shown with nonmetric multidimensional scaling (NMDS) on a Bray–Curtis dissimilarity matrix.

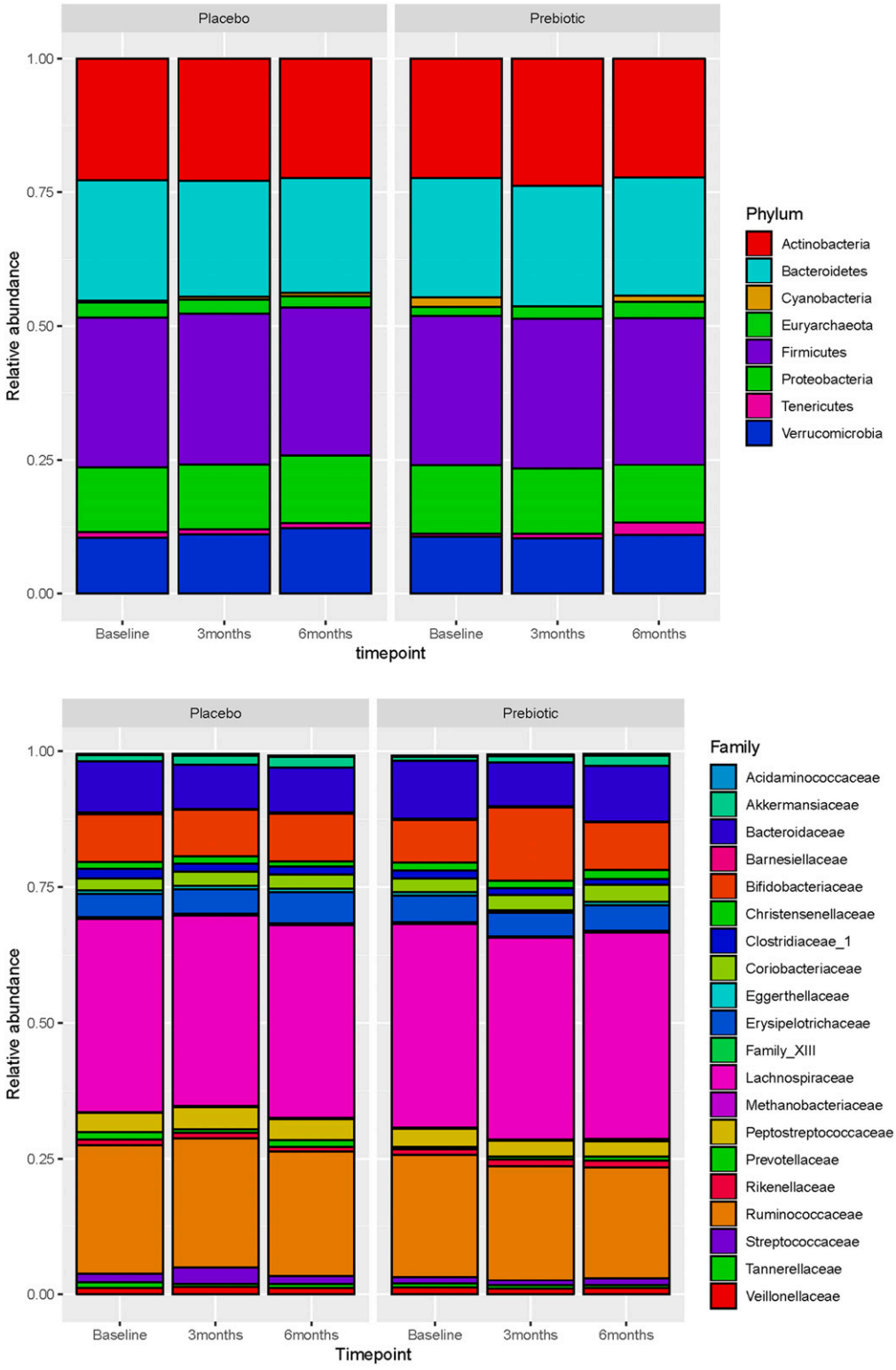


**Figure 4.** Relative abundance plots of the gut microbiota at the phylum and family level in children with T1D at baseline, after consuming placebo or prebiotic for 3 mo, and after a 3-mo washout (6 mo).

the prebiotic during the 3-month treatment period, the preservation of C-peptide in the prebiotic group is a promising clinical marker of pancreatic $\beta$-cell function (38). It is likely we did not see a change in A1C due to the small sample size and relatively short duration of the intervention. Further longer-term studies with prebiotic intervention are needed to demonstrate whether improvement in C-peptide persists and translates into improved overall glycemic control as measured by A1C. In terms of potential mechanisms, we previously showed

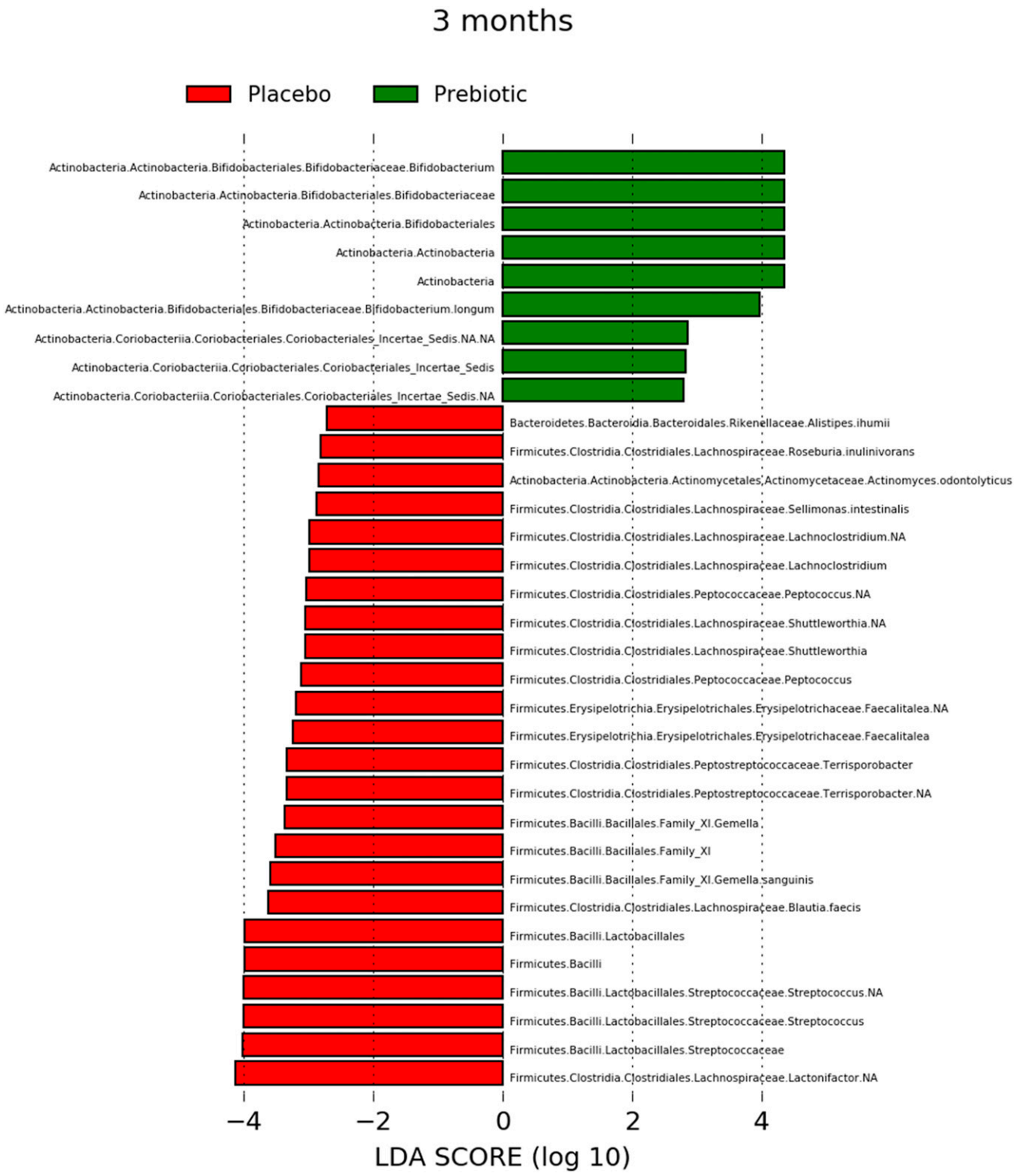


**Figure 5.** LEfSe describing the greatest differences between bacterial groups in children with T1D after consuming placebo or prebiotic for 3 mo. LDA, linear discriminant analysis.

that in diabetic NOD mice (a mouse model of T1D), the prebiotic oligofructose improved insulin sensitivity and increased $\beta$-cell proliferation rate and pancreatic insulin content (39). Mice treated with oligofructose also had increased fecal *Bifidobacterium* abundance (39). Cani *et al.* (6) showed that endotoxemia or the translocation of LPS to host circulation was significantly negatively correlated with *Bifidobacterium* abundance in mice fed a high-fat diet. Feeding oligofructose to the mice on the high-fat diet significantly and positively correlated with improved glucose tolerance and glucose-induced insulin secretion (6). These studies suggest that prebiotics may act

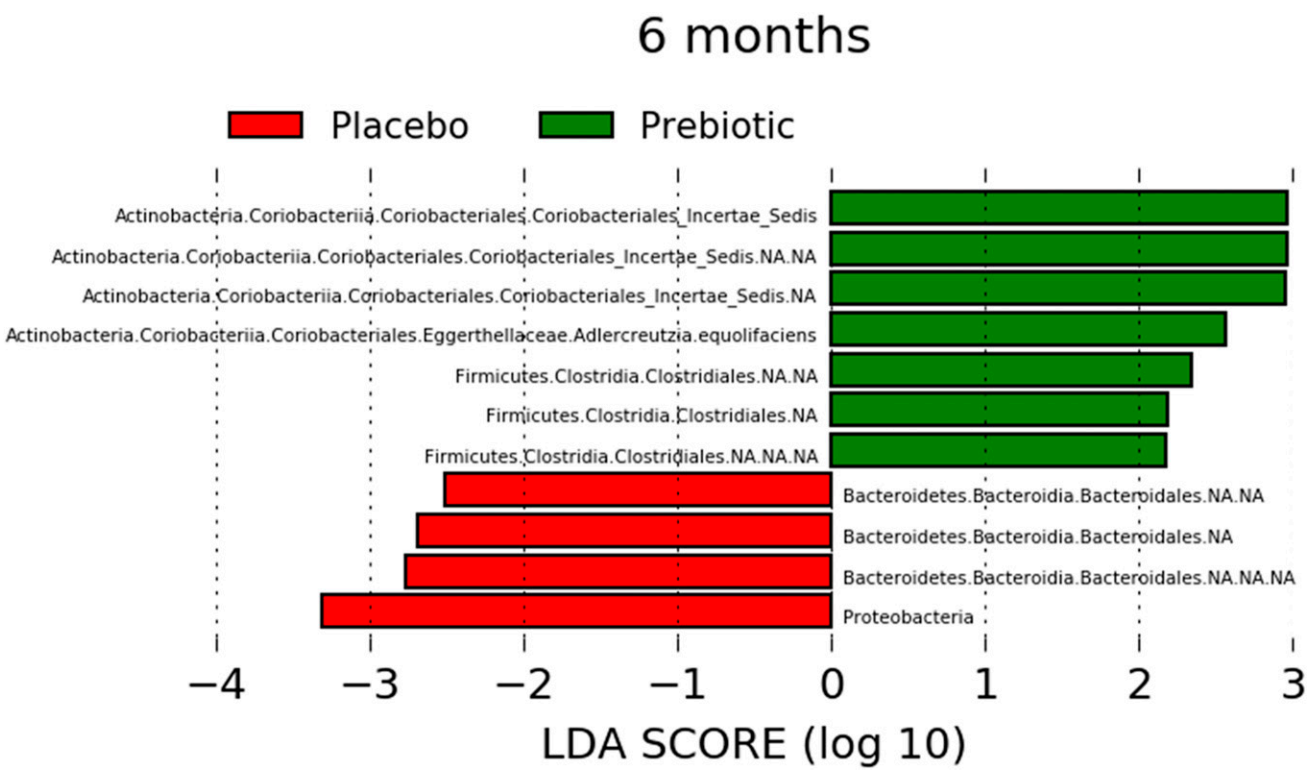


**Figure 6.** LEfSe describing the greatest differences between bacterial groups in children with T1D after a 3-mo washout from consuming placebo or prebiotic (6 mo). LDA, linear discriminant analysis.

on both the pancreatic β-cells directly and modify gut microbiota to increase insulin secretion and reduce insulin resistance, both contributing to lower blood glucose.

A significant body of literature supports the potential role of microbiota, intestinal permeability (9, 14, 16), and mucosal immunity in the pathogenesis of T1D (10, 15). The intestinal mucosal barrier is made up of many cell types, including epithelial cells, the major absorptive cells (9, 40). Our gastrointestinal system provides the largest surface area for interaction with the external environment, and it carries out important immune functions as it constantly "samples" antigens that are present in our gastrointestinal tract; it is also a barrier to exclude harmful pathogens and toxins. Murri *et al.* (41) showed that children with established T1D have different gut microbiota from children without T1D. *Bifidobacterium* has been shown to be underrepresented in children with T1D compared with healthy controls (42, 43). Additionally, proinflammatory cytokines and the endotoxin LPS are increased in T1D (42). This inflammatory response is likely at least in part due to the increased intestinal permeability seen in children with T1D (42).

In animal models, the spontaneously diabetic BB rats and the NOD mice have increased intestinal permeability in comparison (12, 44–46) with their nondiabetic controls. Furthermore, intestinal permeability increases as NOD mice progress from prediabetes to diabetes (45). Importantly, human studies showed that in comparison with healthy individuals, those with β-cell autoimmunity or established T1D have impaired epithelial barrier function with increased passage of antigen through the paracellular pathway (9, 10, 13, 15, 47). On a structural basis, patients with T1D have altered height and thickness of microvilli, abnormal intracellular structures with enlarged intercellular space between enterocytes, and abnormal tight junction domains (35, 48). Hence, there are both functional and structural differences in intestinal epithelial integrity between healthy, prediabetic, and individuals with established T1D. It is hypothesized that the increased gut permeability allows continued exposure to antigens that contribute to aspects of the immune dysregulation observed in T1D (2).

Many studies to date have focused on the influence of diet and gut microbiota on intestinal permeability. NOD mice fed a gluten-free diet had a 50% reduction in diabetes rate (49) whereas providing a gluten-free diet to the pregnant mother is sufficient to reduce intestinal inflammation and diabetes rate in the offspring (50). When diabetes-prone BB rats were fed a hydrolyzed casein diet, both β-cell autoimmunity and intestinal permeability decreased, with increased expression of tight junction proteins (51). Prebiotics have been shown to reduce gut permeability through a mechanism that involves GLP-2, a gut trophic factor (7). In addition to diet, the gut microbiota also affects intestinal permeability (52). In both diabetes-prone BB rats and NOD

**Table 4. Correlations Within the Prebiotic Group**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Baseline | | | | | | | | |
| 1. IL-6 | 1.000 | | | | | | | |
| 2. TNF-$\alpha$ | 0.793[a] | 1.000 | | | | | | |
| 3. C-peptide | 0.432 | 0.273 | 1.000 | | | | | |
| 4. Lac/Man | −0.196 | −0.200 | −0.120 | 1.000 | | | | |
| 5. IFN-$\gamma$ | 0.397 | 0.252 | 0.475 | −0.472 | 1.000 | | | |
| 6. IL-10 | 0.331 | 0.179 | 0.257 | −0.487[b] | 0.343 | 1.000 | | |
| 7. A1C | −0.102 | 0.172 | −0.336 | 0.366 | −0.363 | −0.261 | 1.000 | |
| 8. *Terrisporobacter* | 0.202 | 0.217 | −0.252 | 0.370 | −0.311 | −0.525[b] | 0.185 | 1.000 |
| 3 mo | | | | | | | | |
| 1. IL-6 | 1.000 | | | | | | | |
| 2. TNF-$\alpha$ | 0.825[a] | 1.000 | | | | | | |
| 3. C-peptide | 0.407 | 0.267 | 1.000 | | | | | |
| 4. Lac/Man | −0.16 | 0.052 | −0.163 | 1.000 | | | | |
| 5. IFN-$\gamma$ | 0.680[a] | 0.669[a] | 0.174 | 0.012 | 1.000 | | | |
| 6. IL-10 | 0.268 | 0.228 | −0.140 | −0.133 | 0.393 | 1.000 | | |
| 7. A1C | −0.033 | 0.224 | −0.476 | 0.628[a] | 0.154 | 0.021 | 1.000 | |
| 8. *Terrisporobacter* | 0.053 | 0.076 | −0.484[b] | 0.093 | −0.113 | −0.200 | 0.122 | 1.000 |
| 6 mo | | | | | | | | |
| 1. IL-6 | 1.000 | | | | | | | |
| 2. TNF-$\alpha$ | 0.700[a] | 1.000 | | | | | | |
| 3. C-peptide | 0.490 | 0.206 | 1.000 | | | | | |
| 4. Lac/Man | −0.218 | −0.026 | −0.203 | 1.000 | | | | |
| 5. IFN-$\gamma$ | 0.649[a] | 0.437 | −0.070 | −0.234 | 1.000 | | | |
| 6. IL-10 | 0.394 | 0.342 | 0.029 | 0.262 | 0.349 | 1.000 | | |
| 7. A1C | −0.425 | 0.082 | −0.668[a] | 0.301 | −0.142 | −0.047 | 1.000 | |
| 8. *Terrisporobacter* | 0.116 | −0.73 | −0.58 | −0.122 | 0.007 | −0.449 | 0.007 | 1.000 |

[a]Significant at $P \leq 0.01$.

[b]Significant at $P \leq 0.05$.

mice, manipulation of gut microbiota by antibiotic treatment or fecal transfer leads to altered microbiota composition and changes diabetes incidence (1, 17, 18, 53, 54). A potential mechanism that links an altered healthy microbiota, or "dysbiosis," to diabetes progression is the effects of bacterial fermentation products on intestinal epithelial permeability. The dysbiotic microbiota is often characterized by a reduction in the ratio of butyrate-producing bacteria to Gram-negative bacteria. Butyrate contributes to intestinal epithelial integrity and inhibits inflammation (55, 56). A reduction in butyrate leads to local inflammation in the gut epithelium and loss of tight junction barrier function. In BB rats, administration of butyrate decreased colonic permeability and delayed diabetes onset (57). Prebiotics have been shown to increase butyrate concentrations (58) even though the bacteria that are chiefly increased by prebiotics, *Bifidobacterium*, do not produce butyrate themselves. This increase likely occurs as a result of bacterial cross-feeding (59), and although we did not measure fecal SCFA concentrations, it is possible that the prebiotic administered and the significant increase in relative abundance of *Bifidobacterium* in our prebiotic group could explain improvements in this group over the placebo. In addition to the plausible beneficial role of *Bifidobacterium* in this study, it is interesting to consider the significant correlation between *Terrisporobacter* and both C-peptide and IL-10. Although very little is currently known about *Terrisporobacter*, it has been linked to oxidative stress and inflammation in preterm infants fed formula vs human milk (60), which fits with our data showing a negative correlation with the anti-inflammatory cytokine IL-10, and is interesting to consider in terms of its negative correlation with C-peptide.

These studies suggest that gut leakiness may be modified by dietary factors (61). Our study showed that one third of diabetes patients have a Lac/Man ratio >0.03 at baseline, which is abnormal. In comparison with the placebo group, the group who received prebiotic had improved gut permeability. More importantly, we observed a positive correlation between A1C and the Lac/Man ratio, suggesting that reducing the Lac/Man ratio may reduce A1C, and that intestinal permeability is a modifiable risk factor. Because there is wide variability in intestinal permeability among patients and it takes time for prebiotics to alter gut microbiota composition, which then may influence intestinal permeability, a larger study with prolonged duration of prebiotic intervention will be necessary to determine the magnitude and duration of treatment

**Table 5. Correlations Within the Placebo Group**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Baseline | | | | | | | | |
| 1. IL-6 | 1.000 | | | | | | | |
| 2. TNF-$\alpha$ | 0.374 | 1.000 | | | | | | |
| 3. C-peptide | 0.045 | −0.96 | 1.000 | | | | | |
| 4. Lac/Man | 0.076 | −0.212 | −0.297 | 1.000 | | | | |
| 5. IFN-$\gamma$ | 0.609[a] | 0.374 | 0.097 | −0.214 | 1.000 | | | |
| 6. IL-10 | 0.229 | 0.481[b] | −0.127 | 0.238 | 0.355 | 1.000 | | |
| 7. A1C | −0.312 | 0.045 | −0.385 | 0.237 | −0.073 | 0.151 | 1.000 | |
| 8. *Terrisporobacter* | 0.211 | 0.210 | −0.459[b] | 0.083 | 0.102 | −0.011 | −0.264 | 1.000 |
| 3 mo | | | | | | | | |
| 1. IL-6 | 1.000 | | | | | | | |
| 2. TNF-$\alpha$ | 0.265 | 1.000 | | | | | | |
| 3. C-peptide | −0.093 | −0.477[b] | 1.000 | | | | | |
| 4. Lac/Man | 0.009 | 0.225 | −0.237 | 1.000 | | | | |
| 5. IFN-$\gamma$ | 0.176 | 0.271 | −0.385 | 0.228 | 1.000 | | | |
| 6. IL-10 | −0.105 | 0.326 | 0.036 | 0.393 | 0.455[b] | 1.000 | | |
| 7. A1C | −0.003 | 0.440[b] | −0.466[b] | 0.465[b] | 0.347 | 0.340 | 1.000 | |
| 8. *Terrisporobacter* | 0.037 | −0.011 | −0.030 | −0.392 | −0.185 | −0.338 | −0.405 | 1.000 |
| 6 mo | | | | | | | | |
| 1. IL-6 | 1.000 | | | | | | | |
| 2. TNF-$\alpha$ | 0.290 | 1.000 | | | | | | |
| 3. C-peptide | 0.116 | −0.376 | 1.000 | | | | | |
| 4. Lac/Man | 0.231 | 0.137 | −0.535[b] | 1.000 | | | | |
| 5. IFN-$\gamma$ | 0.302 | 0.556[b] | −0.004 | 0.253 | 1.000 | | | |
| 6. IL-10 | −0.203 | 0.372 | −0.353 | 0.340 | 0.375 | 1.000 | | |
| 7. A1C | −0.194 | 0.337 | −0.567[a] | 0.519[b] | 0.146 | −0.030 | 1.000 | |
| 8. *Terrisporobacter* | −0.216 | −0.096 | −0.420 | −0.159 | −0.333 | −0.234 | 0.110 | 1.000 |

[a]Significant at $P \leq 0.01$.

[b]Significant at $P \leq 0.05$.

effects. Additionally, future studies would benefit from assessing dietary intake in participants, as changes in dietary habits during the course of the study could affect the outcomes observed in the present study.

**Table 6. Self-Reported Adverse Effects for Each Group**

| | Prebiotic (N = 17) | Placebo (N = 21) | *P* Value |
|---|---|---|---|
| No. of subjects reporting gastrointestinal side effects | | | |
| Baseline | 0/17 | 4/21 | 0.057 |
| 3 mo | 1/17 | 1/21 | 0.878 |
| 6 mo | 2/17 | 2/21 | 0.823 |
| No. of self-reported nonsevere hypoglycemia in past week, mean (SD) | | | |
| Baseline | 5.68 (8.38) | 2.02 (2.33) | 0.064 |
| 3 mo | 4.06 (4.02) | 2.53 (2.58) | 0.170 |
| 6 mo | 2.94 (1.94) | 1.71 (1.85) | 0.054 |
| No. of self-reported episodes of severe hypoglycemia in past 3 mo | | | |
| Baseline | 0/17 | 0/21 | — |
| 3 mo | 1/17 | 0/21 | 0.260 |
| 6 mo | 1/17 | 2/21 | 0.679 |
| No. of subjects that self-reported DKA in past 3 mo | | | |
| Baseline | 1/17 | 4/21 | 0.233 |
| 3 mo | 1/17 | 1/21 | 0.878 |
| 6 mo | 0/17 | 1/21 | 0.362 |

A $\chi^2$ test was used to compare categorical variables, and a *t* test was used for continuous variables.

In the present study, we aimed to determine whether treatment with prebiotics could decrease intestinal permeability, which could in turn decrease endotoxemia and reduce insulin resistance, potentially leading to improved glycemic control. Although, no significant differences were seen in A1C, there was higher C-peptide and an improvement in intestinal permeability in the prebiotic group. Prebiotic supplementation, specifically oligofructose-enriched inulin, is a potentially novel, inexpensive, low-risk treatment addition for T1D that may improve glycemic control. Further larger-scale trials are needed.

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***Author Contributions:*** J.H., R.A.R., J.M., and C.H. developed the study protocol. J.H., H.V., A.C.N., A.S., R.A.R., J.M., and C.H. collected and analyzed the data. J.H., R.A.R., and C.H. prepared the manuscript. All authors reviewed and

critically appraised the manuscript. All authors read and approved the final version of this manuscript.

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***Disclosure Summary:*** R.A.R. has received honoraria from Beneo GmbH. The remaining authors have nothing to disclose.

***Data Availability:*** The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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