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Research Article

OZ101, an oligofructose prebiotic, may prolong sulphonylurea efficacy in patients with type 2 diabetes: a pilot study

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Abstract

Objectives: The sulphonylurea class of anti-diabetes drugs lose efficacy over time due to progressive beta-cell failure. Their long-term use may exacerbate gut microbial dysbiosis as they are derivatives of sulphonamide antibiotics. We conducted a pilot study to test the hypothesis that OZ101, administered as adjunctive prebiotic therapy, improves beta-cell function and glycaemic control in sulphonylurea-treated patients.

Materials and Methods: Subjects with type 2 diabetes on sulphonylurea monotherapy (n=30) were randomized in a 24-week parallel dose range-finding study to either continue receiving their usual sulphonylurea-only treatment or add a thrice-daily regimen of 13.5 or 27 g/d doses of OZ101. HOMA-B, glycaemic parameters after 12 hours fasting, and glucose area under the curve (AUC; over 240 minutes) after intake of a pre-defined calorie milkshake were collected at baseline and 24 weeks.

Results: Over 24-weeks, control subjects on sulphonylurea-only showed a decline in beta-cell function (35.54% decrease in HOMA-B from baseline, p = 0.01), whereas subjects taking sulphonylurea+13.5 g/d OZ101 improved (22.9% increase in HOMA-B from baseline, p = 0.031). There was a 0.95% (10 mmol/mol) difference in HbA1c (p = 0.047) and 607 mmol/l*240min AUC (p = 0.039) in favour of the sulphonylurea +13.5g/d OZ101 compared with control group. HbA1c and AUC were not altered in subjects treated with sulphonylurea+27 g/d OZ101 compared with the control group. Microbiome profiling suggested reciprocal relationships in beneficial versus detrimental bacteria between control and treatment groups.

Conclusions: Adjunctive intake of 13.5g/d OZ101 in patients on sulphonylurea therapy was safe, well-tolerated and associated with improved beta-cell function and stabilization of glycaemic control over 24 weeks. Absence of similar response for the 27 g/d OZ101 group may relate to changes in gut microbiome profiles. Future studies will determine the mechanistic link between OZ101 therapy, changes in gut microbiome, and metabolic responses.

Key words: type 2 diabetes, sulphonylurea, prebiotic, microbiome dynamics, beta-cell function, glycaemic control.

1. Introduction:

Loss of beta-cell function with reduced insulin secretory capacity and inability to compensate for insulin resistance are fundamental to the development and progression of Type 2 Diabetes (T2D) [1], [2]. Continued decline in beta-cell function is a key contributing factor to hyperglycaemia and further evolution of T2D and its associated complications. Whilst beta-cell deterioration can initially be ameliorated with lifestyle interventions and certain drugs [3], if early glycaemic control is not achieved, a cascade of events may trigger further worsening of beta-cell function over time. These may include glucotoxicity, lipotoxicity, cytokine imbalance, oxidative and endoplasmic reticulum stress and insulin resistance [4].

Beta-cell deterioration may limit the effectiveness and durability of some anti-diabetes agents. Specifically, sulphonylurea (SU) lose efficacy over time particularly in patients with lower beta-cell function [5]. Moreover, compared to patients prescribed metformin or rosiglitazone, more patients on SU therapy lost glycaemic control over 5-years monotherapy (21% Metformin v 15% rosiglitazone v 34% SU) [6], suggesting that SU therapy also specifically contributes to beta-cell deterioration.

The reasons for the enhanced rates of beta-cell failure with SU are much debated [3], [7]. Given that they are derivatives of sulphonamide antibiotics and possess bacteriostatic activities [8]–[11], long-term use might disturb gut microbiome. Gut microbiome diversity plays an important role in host metabolism and potentially protects against development of T2D [12], [13]. In contrast, globally ~90% of diabetes cases studied show gut microbiome dysbiosis [14] which may contribute to the progressive nature of beta-cell dysfunction, insulin resistance and T2D. Given that certain bacteria are positively associated and others negatively associated with progression of T2D [15], it might be possible to identify beneficial gut bacteria that promote long-term glycaemic control.

We speculate that the antibiotic characteristics of SU may negatively affect gut microbiome, resulting in (i) further deterioration of beta-cell function, (ii) increased risk of unwanted side effects such as hypoglycaemia and weight gain and, ultimately, (iii) loss of anti-glycaemia effectiveness. We hypothesize that the antibiotic effects of SU on gut microbiome may be ameliorated through adjunctive intake of prebiotics, addition of which may limit loss of beneficial bacteria [15]. We have tested this hypothesis in a phase 2 clinical trial, in a resource constrained area in India, in patients on SU monotherapy by measuring beta-cell function, glycaemic parameters, diabetes related biomarkers and microbiome profiles before and after 24 weeks of prebiotic intervention.

2. Materials and Methods:

2.1 Design

We performed a pilot, proof of concept and dose range finding, randomized controlled clinical trial at the Bangalore Diabetes Centre, Bangalore, India and Health Centre Point, Kellyville, NSW, Australia. It was conducted according to the Declaration of Helsinki and ICH-GCP guidelines and approved by the Medisys Clinisearch Ethical Review Board, Medisys Clinisearch India Private Limited, Bangalore Diabetes Centre, Kalyan Nagar, Bangalore, India and Bellberry Limited Human Research Ethics Committee, Adelaide, South Australia, Australia. The study was registered at the Australian New Zealand Clinical Trial Registry (ACTRN12614000836639) with Universal Trial Number U1111-1158-9653. OpenClinica Enterprise Edition was employed to electronically capture and manage clinical data (OpenClinica LLC, Waltham, MA). A clinical research organization (George Clinical Pty Ltd, Newtown, NSW, Australia) monitored this clinical trial and compared the electronically captured data with the paper source documents to monitor and confirm data accuracy on a monthly basis. Source data verification was conducted at 100% for the primary and secondary endpoints.

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2.2 Subjects

Patients with T2D on SU monotherapy were recruited. Eligibility criteria: ability to give written informed consent; age 25-65 y; BMI 25 - 40 kg/m2, diagnosed with T2D according to accepted criteria (American Diabetes Association) with duration 1-10 years; on SU monotherapy, at or below maximum tolerated dose for at least 6 months prior to study entry; fasting serum glucose (FSG) >7 mmol/L and HbA1c between 7-10%; no adjustment in SU monotherapy during the previous 12 weeks and during this time had no change in other medications which could affect glucose metabolism such as glucocorticoids, statins, diuretics, beta blockers, ACE inhibitors, angiotensin receptor blockers and oral beta agonists.

Exclusion criteria: excessive consumption of uncooked oligofructose/inulin containing products within 4 weeks prior to randomization or likely to consume excessive amounts during the trial (a list of these products with daily intake limits was provided to each enrolled patient); taking probiotic supplements 4 weeks prior to randomization or planning to do so during the trial; allergy to oligofructose/inulin containing products; taking any other non-SU oral or parenteral anti-diabetes medication; planning to fast for more than 7 consecutive or non-consecutive days during the trial; severe hepatic dysfunction; significant neuropathy or nephropathy; serious cardiovascular disease; history of heart failure; cancer; inflammatory bowel disease; planned surgical operation involving general anaesthesia; other concomitant systemic diseases.

2.3 Intervention

Thirty-nine patients provided written informed consent. After exclusion of 4 patients who did not meet the inclusion/exclusion criteria, 35 patients were randomly assigned to one of three study arms. 5 patients withdrew their consent prior to OZ101 dosing leaving 30 remaining patients. They were asked to maintain their lifestyles including food intake and investigators were requested not to provide any advice which might lead to a lifestyle change during the trial. After 4 weeks of screening, patients randomized to arm 1 continued their usual SU intake for the entire trial. Patients in arms 2 and 3 were prescribed thrice daily OZ101 tablets, at 13.5 g/d (9 x 1.5 g OZ101 tablets) and 27 g/d (18 x 1.5 g OZ101 tablets) respectively, whilst continuing with their usual SU dose (see Figure 1). The sponsor's proprietary OZ101 tablet contained 94% food-grade oligofructose and 6% Therapeutic Goods Administration-approved excipients.

2.4 Compliance

Each patient visited the study centre every 4 weeks to receive a monthly supply of OZ101 and for assessment of tablet compliance. Compliance was recorded by the patient completing a diary with professional assessment at each visit. At such visits the pharmacist, or other suitably qualified person, counted the unused portion of the blister packs. Compliance with OZ101 was based on evidence of at least 75% of the prescribed/intended amount being used during the 4-weekly treatment period.

2.5 Outcome measures

Changes were compared in (i) haemoglobin A1c (HbA1c), (ii) fasting serum glucose (FSG) and (iii) 2 and 4 hours post-prandial glucose (PPG) after a mixed meal challenge in patients treated for 24 weeks with OZ101 plus SU versus SU alone. Fasted patients drank a standard milkshake containing carbohydrate, fat and protein with pre-defined calories to measure PPG at 2 and 4 hours. Serum glucose values at three points (12-hours fasting, 2 and 4 hours post milk shake intake) were used to measure the glycaemic area under the curve (AUC) for each patient at baseline and 24 weeks post treatment. HOMA-B was calculated based on fasting plasma insulin and serum glucose from the same venepuncture using the equation: HOMA-B = (20 x insulin in μU/ml) / (glucose in mmol/L - 3.5) [16], [17].

Other diabetes related biomarkers such as levels of plasma insulin, C-peptide, glucagon, total glucagon-like peptide 1(GLP-1), active GLP-1, and adiponectin were also measured. Patients' stools were collected before and after 24 weeks prebiotic intervention to measure changes in the gut microbiome profiles. Other study parameters included comparison of body weight and body mass index (BMI). Safety aspects included

evidence of mild, severe and nocturnal hypoglycaemia, biochemistry, lipid profile, haematology and urine tests.

2.6 Visits

Each patient visited the study investigator twelve times. These included discussions of the trial and taking consent at the pre-screening visit. Clinical assessments and venepuncture were performed at the screening visit, baseline visit (randomisation), and 12- and 24-weeks post intervention. During the remaining intermediate visits, the investigator assessed any adverse events and concomitant medications. Patients had been given a document wallet that contained a copy of patient information and consent form, 3 diaries, 2 questionnaires, patient card, OZ101 handling and storage information. The diaries and questionnaires were designed to collect information about meal intake, exercise status and adverse events.

Standard duty of care procedures were undertaken at each visit. If a patient discontinued for any reason, a "Discontinuation Visit" was scheduled and a case report form completed to capture the reason(s) for discontinuation as well as provision of suitable follow-up. In all patients a "Follow-up Visit" was scheduled 4 weeks after the last dose to ensure patients safety and well-being.

2.7 Specimen Collection

Blood was collected into Beckton Dickinson (Franklin Lakes, NJ) vacutainer® tubes. For glucose, blood was collected into sodium fluoride tubes. For measurement of clinical chemistry parameters including lipid profiles, blood was collected into tubes with gel clot activator + serum separator. For HbA1c, haematology parameters and adiponectin blood samples were collected into K2EDTA tubes. For glucagon, C-peptide, insulin, GLP-1 total and GLP-1 active, blood was collected into P-800 tubes containing protease inhibitors. Urine was collected into sterile tubes. All specimens were prepared according to manufacturer's instructions and either used immediately or flash frozen and kept at -75^{0}C until use. HbA1c, glucose, clinical chemistry, haematology and lipid profiles were immediately measured. Frozen specimens were shipped in dry ice to AssayGate Inc. (Ijamsville, MD) to measure adiponectin, glucagon, C-peptide, insulin, total GLP-1 and active GLP-1. Stool samples were collected in OMNIgene®•GUT to stabilize microbial DNA for gut microbiome profiling (Cat # OMR-200, DNA Genotech, ON, Canada). Collected samples were transferred into cryogenic tubes, flash frozen and transported in dry ice to the USA (Diversigen Inc., New Brighton, MN) for sequencing.

2.8 Metagenomic Analysis of Fecal Microbiome

DNA samples were extracted with MO Bio PowerSoil Pro automated for high throughput on QiaCube (Qiagen Inc. Valencia, CA) and were quantified with Quant-iT Picogreen dsDNA Assay (Invitrogen, Inc. Carlsbad, CA). Libraries were prepared with a procedure adapted from the Nextera Library Prep kit (Illumina Inc., San Diego, CA) and were sequenced on an Illumina NextSeq (Illumina Inc., San Diego, CA). Operational Taxonomic Units (OTUs) were picked by aligning DNA sequences to a curated database containing all representative genomes in RefSeq for bacteria with additional manually curated strains (Venti). Alignments were made at 97% identity against all reference genomes. Every input sequence was compared to every reference sequence in CoreBiome's Venti database using fully gapped alignment with BURST. Samples with fewer than 10,000 sequences were also discarded. OTUs accounting for less than one millionth of all species-level markers and those with less than 0.01% of their unique genome regions covered (and < 1% of the whole genome) were discarded. The number of counts for each OTU was normalized to the average genome length. The normalized and filtered tables were used for all downstream analyses. Logarithmic ratios of counts for each bacterial family were calculated by dividing Log count post 24 weeks prebiotic intervention to Log counts at baseline. Percent deviation from 1 was used to compare the changes in microbial families at each study arm.

2.9 Statistical Analysis

A computer program stratified randomization list was prepared based on four strata as follows: (i) FSG < 10 mmol/L and diabetes duration of less than 5 years, (ii) FSG < 10 mmol/L and diabetes duration of equal or more than 5 years, (iii) FSG ≥ 10 mmol/L and diabetes duration of less than 5

years, (iv) FSG ≥ 10 mmol/L and diabetes duration of equal or more than 5 years. An intention-to-treat analysis was adopted, and the missing data were imputed with baseline values for a conservative estimate (i.e., no change). All the data were checked for normal distribution. Normally distributed variables were described using mean and standard deviation, while skewed variables were described using medians (Q1, Q3). Descriptive statistics were presented as the mean standard deviation or median with interquartile range (IQR). The changes in primary outcomes (HbA1c, FSG and PPG) in arm 2 and arm 3 were separately compared to arm 1 (control) and were analysed using two sample t-test. Baseline values were added as covariate. The secondary outcomes were analysed using a bootstrapped quantile regression model to compare the study groups at week 24.

3. Results:

3.1 Study Participants

A CONSORT flowchart [18] of progression of the participants through the trial and baseline characteristics of randomized participants are shown in Figure1 and Table 1, respectively. Screening started in July 2018 with last visit November 2019 - 80% of participants completed the study. Supplementary Table 1 shows the number of patients with available data to conduct an intention-to-treat analysis. Missing data for two patients, who attended 12 but not 24-week visits, (one control and one from the 27 g/d OZ101 arm) were imputed.

Table 1 shows mean and range of duration of diabetes for each study arm. All patients had been taking SU monotherapy (glibenclamide or glimepiride or glipizide) for at least the past 6-month period prior to the study. Groups were comparable for the type and dose of SU therapy

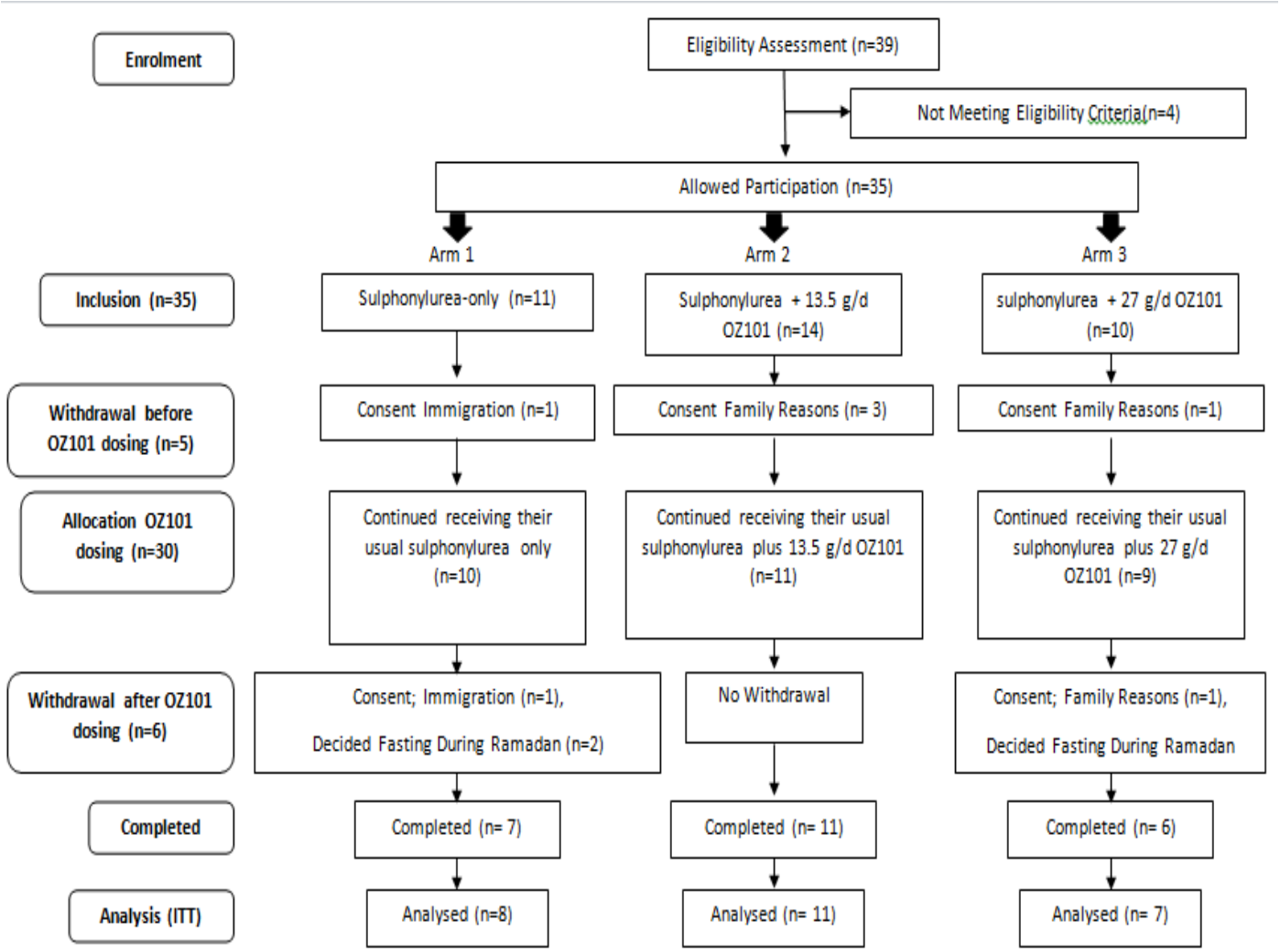

Figure 1: CONSORT flowchart of progress of participants through the trial.

	Sulphonylurea Only	**Sulphonylurea +13.5 g/d OZ101**	**Sulphonylurea +27 g/d OZ101**
	n=11	n=14	n=10
Female ‡	6 (55)	6 (43)	7 (70)
Age (years) §	51 (8)	52 (9)	52 (8)
Years since diabetes diagnosis §	6.1 (2.5)	6.2 (2.4)	6.1 (2.8)
Body Weight (kg) §	75.3 (13.7)	67.5 (7.4)	78.2 (10.3)
Height (cm) §	158.3 (8.1)	156.5 (6.7)	157.9 (9.8)
Body Mass Index (kg/m^2) §	30 (4)	27 (2)	31 (5)
HbA1c (%) §	8.7 (0.9)	8.6 (0.7)	8.5 (0.9)
Fasting serum glucose (mmol/L) §	9.5 (2.3)	9.1 (1.9)	8.5 (2.4)
Triglyceride (mmol/L) ¶	1.52 (1.16, 1.66)	1.74 (1.34, 3.16)	1.64 (1.43, 1.96)
HDL (mmol/L) §	1.05 (0.06)	1.06 (0.05)	1.04 (0.04)
LDL (mmol/L) §	2.70 (0.95)	2.57 (0.49)	2.60 (0.67)
Systolic blood pressure (mmHg) §	123 (7)	126 (6)	127 (7)
Diastolic blood pressure (mmHg) §	80 (4)	79 (5)	82 (5)

Table 1. Baseline characteristics of the study participants by treatment arms. ‡ - n (%), § - mean (SD), ¶ - median (Q1, Q3).

	Sulphonylurea Only	**Sulphonylurea + 13.5 g/d OZ101**	**Sulphonylurea + 27 g/d OZ101**
Baseline	10 (100%)	11 (100%)	9 (100%)
12-weeks follow up	8 (80%)	11 (100%)	7 (78%)
24-weeks follow up	7 (70%)	11 (100%)	6 (67%)
Intention to Treat	8 (80%)	11 (100%)	7 (78%)

Supplementary Table 1: The number of patients with available data for each arm at baseline, 12- and 24-weeks follow ups and Intention to Treat are shown. Missing data for two patients, who attended 12 but not 24-week follow up (one from Sulphonylurea Only and one from the Sulphonylurea +27 g/d OZ101 arm) were imputed.

3.2 HOMA-B and glycaemic control

Compared to baseline, by 24 weeks the SU-only (control) group showed 35.54% reduction in HOMA-B (68±23 to 47±17 µU/mole, p = 0.01), whereas the group taking SU and 13.5 g/d OZ101 showed 22.90% increase in HOMA-B (98±14 to 123±22 µU/mole, p = 0.031) (Figure 2a). This was reflected in an overall difference in HOMA-B between the two groups of 58.44%.

Compared to the baseline values, the AUC^{0-240} was increased by average of 14.91% in the control group (2,852 ± 201 mmol/L*240 min to 3,287 ± 257 mmol/L*240 min). In contrast, there was a 5.31% reduction from the baseline in $AUC0^{-240}$ (2,852 ± 229 mmol/L*240 min to 2,776 ± 168 mmol/L*240 min) in the group taking additional 13.5 g/d OZ101. Overall, the addition of 13.5 g/d OZ101 to SU treatment resulted in 20.21% difference in $AUC0^{-240}$ compared with control (P = 0.039) (Figure 2b)

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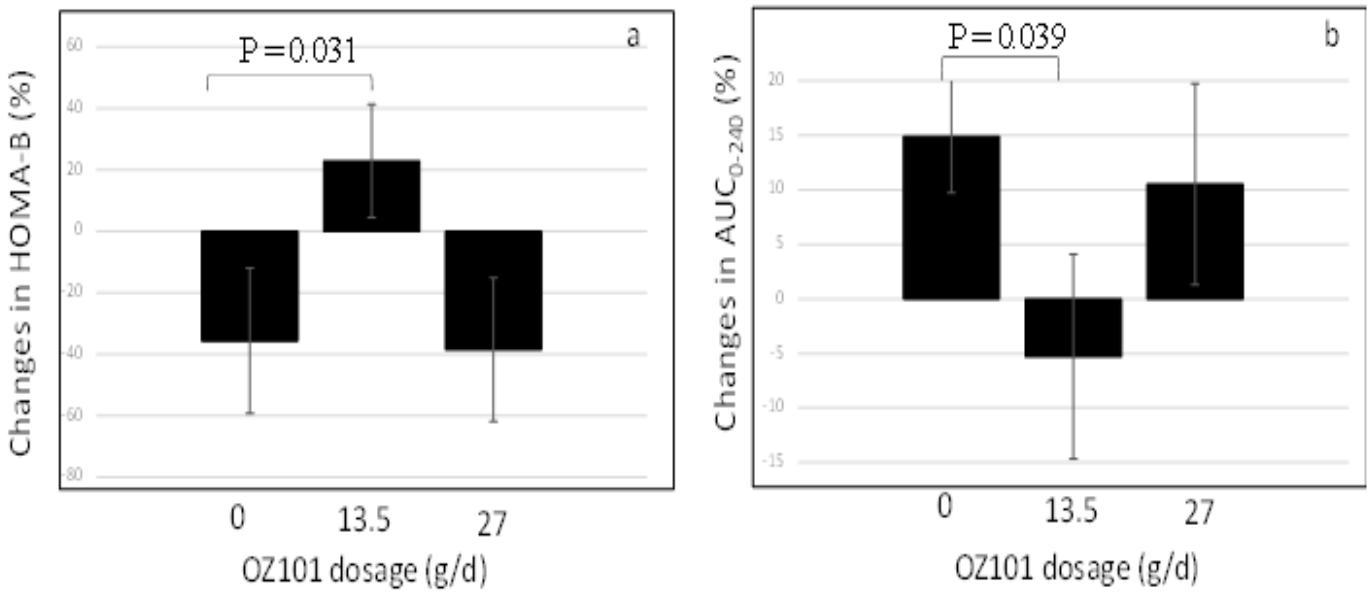


Figure 2: Changes in HOMA-B (a) and $AUC0^{-240}$ min (b) between baseline and 24-weeks post intervention. The box plots represent percentage changes in HOMA-B and AUC in patient on sulphonylurea-only treatment (n=8) and in patients who were given 13.5 g/d (n=11) or 27 g/d (n=7) OZ101 in addition to their usual prescribed sulphonylureas. Error bars represent standard error mean. HOMA-B = homeostasis model assessment of β-cell, AUC = Area under the curve 0 to 240 minutes.

Except for 2h postprandial serum glucose, all other glycaemic parameters were increased from baseline. However, this increase was attenuated by 80-90% in the 13.5 g/d OZ101 treated compared with the control group. By 24 weeks the control group showed 1.18% (12.9 mmol/mol) increase in HbA1c from baseline compared with an increase of 0.23% (2.6 mmol/mol) in 13.5 g/d OZ101 group, a difference of 0.95% (10.3 mmol/mol) (p = 0.047) (Figure 3a).

Similarly, the control group showed a (non-significant) greater rise from baseline to 24 weeks in both PPG (Figure 3b) and FSG (Figure 3c) compared with the group taking adjunctive 13.5g/d OZ101 (PPG: 2.3 increase v 0.7 mmol/l reduction respectively; FSG: 2.3 increase v 0.2 mmol/l increase, respectively). 4-hour PPG showed a similar non-significant trend (increase was 2.1 v 0.10 mmol/l for the control and 13.5g/ day OZ101, respectively) (Table 2) i.e., at page 8).

Adjunctive intake of 27 g/d OZ101 was not associated with any changes compared with the control group in HOMA-B and glycaemic control (Figure 2, and Figure 3).

There were no significant differences either within or between groups in lipid profiles (supplementary Table 2)

Lipid Parameters		Sulphonylurea Only (n=8)	Sulphonylurea +13.5 g/d OZ101 (n=11)	p-value	Sulphonylurea +27 g/d OZ101 (n=7)	p-value
HDL (mmol/L)	Changes from baseline (Mean (SD))	0.007 (0.08)	-0.005 (0.05)		-0.008 (0.06)	
	Differences between control and treatment arms (mean (95% CI))		-0.01 (-0.08, 0.05)	0.70	-0.02 (-0.10, 0.07)	0.70
LDL (mmol/L)	Changes from baseline (Mean (SD))	0.50 (0.87)	0.29 (0.37)		0.09 (0.24)	
	Differences between control and treatment arms (mean (95% CI))		0.21 (-0.83, 0.41)	0.49	-0.41 (-1.27, 0.39)	0.29
Triglycerides (mmol/L)	Changes from baseline (Mean (SD))	0.07 (-0.32, 0.43)	0.09 (-0.14, 0.47)		-0.02 (-0.38, 0.32)	
	Differences between control and treatment arms (mean (95% CI))		0.02 (-0.58, 0.62)	0.94	-0.11 (-0.52, 0.30)	0.58

Supplementary Table 2: Changes in lipid parameters for intention to treat patient groups are shown. No significant differences either within or between groups were observed. Changes for each lipid parameters in study arms from the baseline are presented as Mean ± SD. The differences between control and treatment arms are presented as mean 95% Confidence Intervals.

3.3 Plasma insulin, C-peptide and other parameters

Plasma insulin declined by 13.42% and 26.01% from baseline to 24 weeks in the control (121.85±57.59 pmol/L to 105.50±87.48 pmol/L) and 27 g/d OZ101 (218.51±69.98 pmol/L to 161.68±96.38 pmol/L) arms, respectively. In contrast, the 13.5 g/d OZ101 group exhibited a 49.26% increase (139.32±80.93 pmol/L to 207.95±99.56 pmol/L), representing a 62.68% difference from the control arm (Figure 3d).

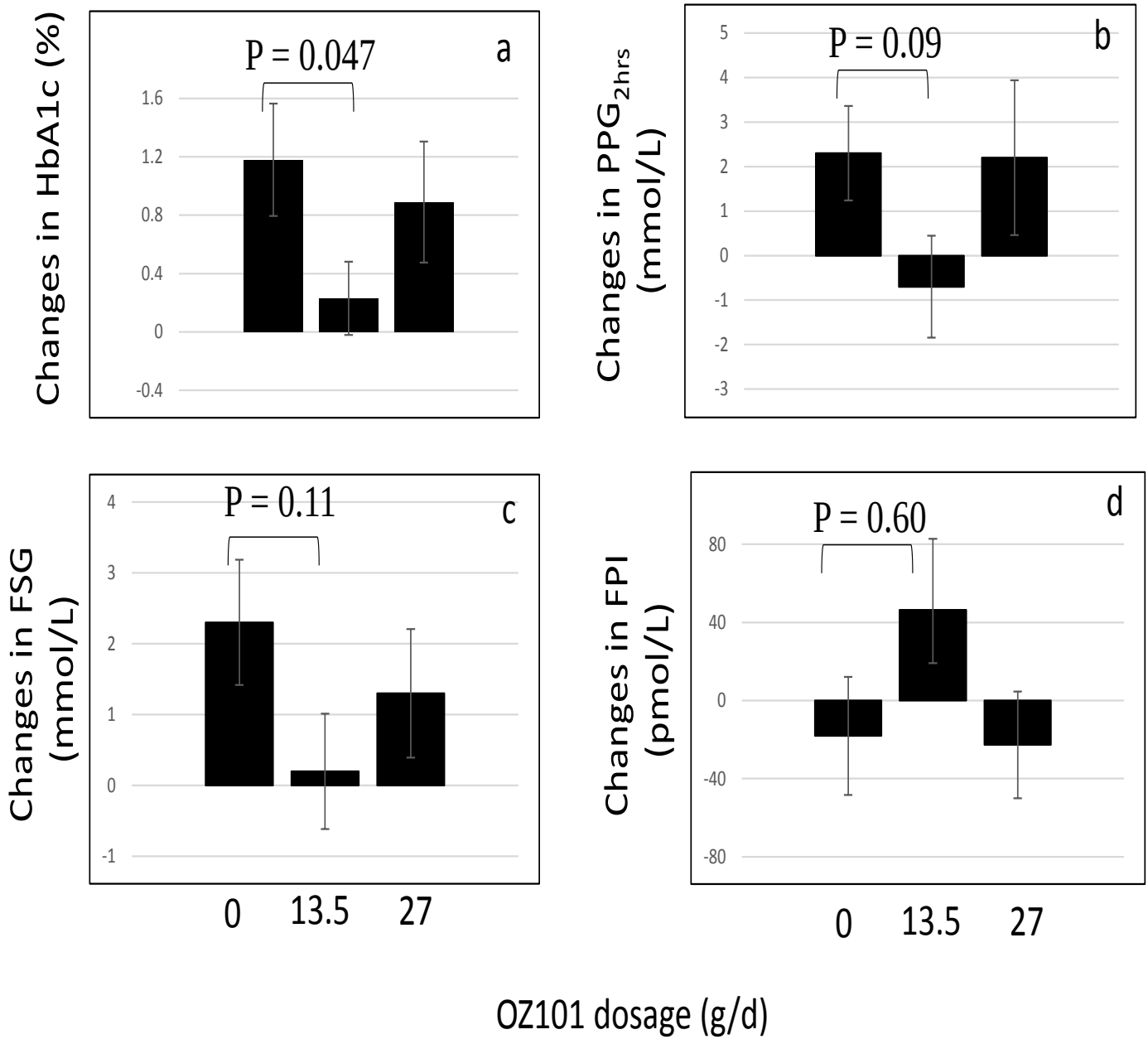


Figure 3: Glycaemic parameters. Changes in HbA1c (a), 2 hours post prandial glucose (b), fasting serum glucose (c), and fasting plasma insulin (d) in study arms from the baseline are presented as Mean ± SEM. The differences between control and treatment arms are presented as mean 95% Confidence Intervals.

C-peptide levels also declined from the baseline by 14.81% (638.31±343.35 pmol/L to 543.76±312.91 pmol/L) and 26.00% (845.11±393.74 pmol/L to 625.35±279.13 pmol/L) in the control and 27 g/d arms, respectively. The 13.5 g/d OZ101 group showed a 3.64% increase (665.48±436.06 pmol/L to 689.70±328.67 pmol/L) in C-peptide, representing an 18.45% difference compared with the control arm (Table 2). Although we observed reciprocal relationships with glycaemic parameters, the changes in insulin and C-peptide were not statistically significant.

There were no significant changes and no differences between the 3 groups for the other biochemical measurements including plasma glucagon, total and active GLP-1 and adiponectin (Table 2).

Diabetes Biomarkers		**Sulphonylurea Only (n=8)**	**Sulphonylurea +13.5 g/d OZ101 (n=11)**	**p-value**	**Sulphonylurea +27 g/d OZ101 (n=7)**	**p-value**
Plasma C-peptide (pmol/L)	Changes from baseline (Mean (SD))	-78.87 (206.3)	-5.83 (398.51)		-258.87 (303.48)	
	Differences between control and treatment arms (mean (95% CI))		73.04 (-274.87, 420.95)	0.66	-180.0 (-492.39, 132.38)	0.23
Plasma Glucagon (log(pg/ml))	Changes from baseline (Mean (SD))	-0.03 (0.33)	-0.14 (0.57)		0.30 (0.74)	
	Differences between control and treatment arms (mean (95% CI))		-0.11 (-0.80, 0.60)	0.74	0.34 (-0.66, 1.33)	0.44
Plasma $GLP1_{total}$ (log(pg/ml))	Changes from baseline (Mean (SD))	-0.13 (0.73)	-0.07 (0.57)		0.36 (0.70)	
	Differences between control and treatment arms (mean (95% CI))		0.06 (-0.78, 0.91)	0.87	0.49 (-0.75, 1.73)	0.37
Plasma $GLP1_{active}$ (log(pg/ml))	Changes from baseline (Mean (SD))	0.02 (1.98)	-0.06 (2.90)		0.50 (1.91)	
	Differences between control and treatment arms (mean (95% CI))		-0.08 (-3.70, 3.54)	0.96	0.48 (-2.89, 3.85)	0.74
Plasma Adiponectin (log(ng/ml))	Changes from baseline (Mean (SD))	1.17 (1.81)	-0.10 (1.28)		-0.21 (0.99)	
	Differences between control and treatment arms (mean (95% CI))		-1.27 (-3.26, 0.72)	0.19	-1.38 (-3.91, 1.14)	0.23
4 hrs Postprandial serum glucose (mmol/L)	Changes from baseline (Mean (SD))	2.1 (3.6)	0.1 (3.9)		1.70 (4.9)	
	Differences between control and treatment arms (mean (95% CI))		-1.9 (-5.6, 1.80)	0.29	0.3 (-5.1, 4.4)	0.88

Table 2: Changes in diabetes biomarkers. Changes in study arms from the baseline are presented as Mean ± SD. The differences between control and treatment arms are estimated using quantile regression and are presented as mean 95% Confidence Intervals.

3.4 Faecal Microbiome Dynamics

Faecal levels of Bifidobacteriaceae, Lactobacillaceae, and Bacteroidaceae families declined by 5.46%, 3.69% and 5.60%, from baseline to 24 weeks in the control arm, respectively. In contrast, these bacterial species increased by 13.67%, 2.82%, and 10.76% in the

13.5 g/d group, representing 19.13% (p = 0.017), 6.51% (p= 0.28), 16.36% (p= 0.30) differences from the control arm, respectively. Similarly, the 27 g/d group exhibited a 16.03%, 7.19%, and 24.39% increase in these bacterial species, representing a 21.49% (p= 0.022), 10.88% (p= 0.30), 29.99% (p= 0.19) differences from the control arm, respectively (Figure 4a-c). In contrast, faecal levels of Streptococcaceae families increased by 8.91% from baseline to 24 weeks in control arm, as opposed to 24.73% and 11.06% reductions, representing 33.64% (p = 0.22) and 19.97% (p = 0.22) differences from the control arm, in 13.5 g/d and 27 g/d arms, respectively (Figure 4d). Similarly, Micrococcaceae and Actinomycetaceae families increased in the control arm but decreased in the treatment arms (supplementary Figure 1). Faecal levels of Lachnospiraceae families declined by 10.65% and 12.24% in control and 13.5 g/d arms but these families of bacteria increased by 6.77% in the 27 g/d arm, representing 17.42% and 19.01% (p = 0.05) differences, respectively (Figure 4e). Similarly, the faecal levels of Ruminococcaceae and Erysipelotrichaceae families declined by 8.40% and 5.61% in control and 13.5 g/d arms but increased by 7.91% in the 27 g/d arm, representing 16.31% (p = 0.033) and 13.52% (p = 0.05) differences, respectively (Figure 4f).

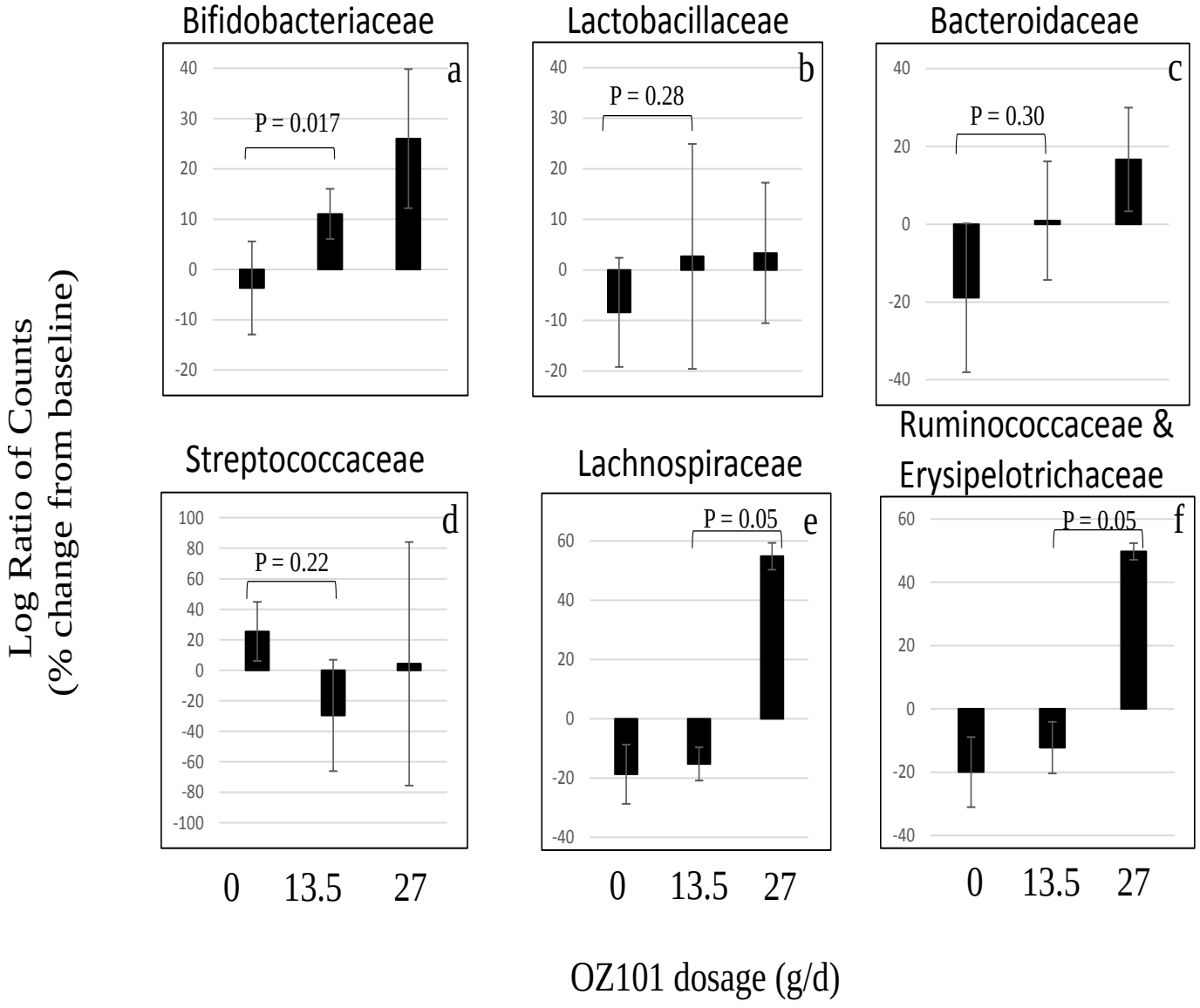
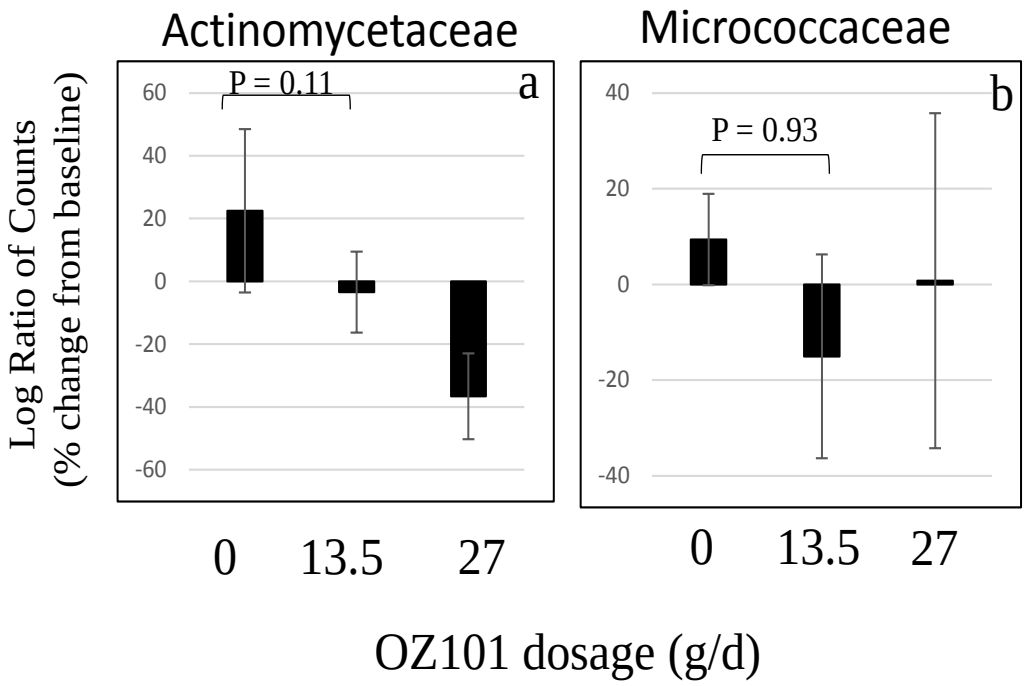


Figure 4: Stool microbial dynamics. Changes in the families of Bifidobacteriaceae (a), Lactobacillaceae (b), Bacteroidaceae (c), Streptococcaceae (d), Lachnospiraceae (e), and Ruminococcaceae/Erysipelotrichaceae (f) in study arms from the baseline are presented as Mean ± SEM.



Supplementary Figure 1. Stool microbial dynamics. Changes in Actinomycetaceae (a), Micrococcaceae (b) in study arms from the baseline are presented as Mean ± SEM.

3.5 Safety assessment

One patient from the control arm had a grade-1 hypoglycaemic episode during a baseline visit (blood glucose 2.1 mmol/l). No other safety or tolerability issues were reported during the course of this study.

4. Discussion:

In this proof of concept and dose finding study we provide preliminary evidence that adjunctive intake of the lower dose of the prebiotic formulation OZ101 may be associated with improvement in HOMA-B (a marker of beta-cell function) and stabilisation of glycaemic control in patients on long term SU therapy. The improvement in HOMA-B and stabilisation of glycaemic control correlated with increases in fasting insulin and C-peptide, although statistically non-significant. In contrast, there was no significant change in other metabolic parameters including plasma glucagon, total GLP-1, active GLP-1, adiponectin and lipids. The higher dose of OZ101 failed to demonstrate the improvements seen with the lower dose.

The hypoglycaemic potential of SU compounds was first recognised in 1942 based on observations that patients prescribed a sulphonamide antibiotic (para-amino-benzene-sulfamido-isopropylthiodiazol, 2254 RP), while treating Salmonella typhi infection, developed very low blood glucose [19]. Four years later it was reported that this group of antibiotic compounds, now known as SU, stimulate insulin release from beta-cells [20]. Since then, three generations of SU class drugs have been developed and prescribed worldwide to hundreds of millions of patients with T2D [21]. SUs commonly provide good blood glucose control at the outset but, over time, lose efficacy [22]. This latter has been attributed to pancreatic beta-cell exhaustion [5], this being a function of the natural history of T2D but becoming more rapidly progressive with the use of SUs [6]. We hypothesized that SU loss of efficacy may be partly due to its antibiotic properties. Indeed, SUs and their derivatives possess bacteriostatic properties and are currently being developed for the treatment of Streptococcus pyogenes [8], Mycobacterium tuberculosis [9], and several other pathogens [10], [11].

We further speculate that the long-term and repeated use of SU may inadvertently promote progressive gut microbiome dysbiosis, which may be overcome by adjunctive intake of prebiotics. This was supported by studies showing that probiotic supplementation to rats with diabetes

increased systemic absorption of gliclazide and reduced blood glucose levels [23]. In contrast to our hypothesis, 12 weeks treatment with gliclazide on patients taking metformin did not change gut microbiome composition [24]. This discrepancy may be due to short term treatment with the SU or prior treatment with metformin, which is known to affect the gut microbiome composition. Nevertheless, current study supports the view that SU-only therapy promotes gut microbiome dysbiosis (see below).

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon [25]. Due to their established beneficial effects on gut microbiota, fructans such as inulin, oligofructose (a hydrolysed form of inulin) and fructo-oligo-saccharide (FOS) are established as water soluble prebiotics [26], [27]. Although several potentially beneficial effects of fructans in healthy subjects have been reported [28], effects on glycaemic control in animal models were inconsistent and clinical trials in humans have been equivocal. Supplementation of oligofructose (5% w/w diet) did not provide any beneficial effects during the development of diet-induced diabetes in rats [29]. Moreover, life-long oligofructose supplementation (10% w/w diet) to healthy rats did not result in changes to FSG at any time point during the study [30]. Clinical trials published between 1984 and 2009 also suggested that fructans such as inulin, oligofructose and FOS do not affect blood glucose control. These trials reported that (i) supplementation of 20 g/d of FOS to healthy [31]–[33], hypercholesterolemic [34], [35] or subjects with T2D [36] did not modify FSG, HbA1c, insulin concentrations and lipid profiles, (ii) supplementation of 15 g/d of oligofructose to patients with T2D in a randomized trial had no significant effect on blood glucose [37], (iii) supplementation of pure inulin to healthy subjects had no effect on FSG [38], and (iv) 6 months supplementation with a mixture of inulin and oligofructose to healthy subjects had no effect on FSG [39]. A systematic review [40] also concluded that consumption of fructans such as inulin, oligofructose and FOS have no significant glucose lowering effects in humans. In contrast, other clinical trials suggested that supplementation with pure inulin [41] and oligofructose [42]–[44] may improve glycaemic indices in female patients taking SU and metformin. A recent review proposed that, amongst studied prebiotics, oligofructose-enriched inulin may improve metabolic and inflammatory biomarkers related to T2D in women [45]–[47].

Since consumption of inulin, oligofructose and FOS provides negligible calories to the host (not digested) and their known primary mechanism of action involves increased beneficial bacteria in the gut, the discrepancies seen in clinical trials may be due to (i) insufficient trial duration, (ii) not taking into consideration the effects of antidiabetes agents or their combination on the host gut microbiome, (iii) or use of maltodextrin or potato starch (with calories equivalent to sucrose) as placebo may have deteriorated glycaemia in control arm patients.

To address the above, in the current study we have: (i) conducted the trial for 6 months to ensure adequate timing for recuperating the gut microbiome; (ii) only included SU monotherapy patients to ensure that other confounding factors such as effects of other drugs on the host microbiome are eliminated; (iii) compared patients continuing SU-only with those taking SU plus adjunctive OZ101. We could not provide placebo OZ101 as we were unable to find/manufacture a placebo molecule similar to oligofructose which provides no calories to the host and has no effect on the gut microbiome.

Our small study provides preliminary evidence to support our hypothesis that OZ101 supplementation at an optimized dose (i.e., 4.5 g thrice daily with meals) synergises with SU drugs to improve HOMA-B and stabilise glycaemic control. As discussed, previous studies reported that generally oligofructose supplementation alone had no effects on glycaemic parameters in animals and humans [29], [30], [39], [40], [31]–[38]. We propose that this discrepancy may be explained by OZ101 providing benefit by ameliorating the negative effects of SUs specifically on the gut microbiome. In this context, the lesser and non-significant effects on SU-mediated glycaemic control with higher dose OZ101 could be due to

further (unwanted) changes in gut microbiome dynamics due to excessive intake of OZ101.

Gut microbiome dynamics and their role in host metabolism and glycaemic control are not well understood. A recent review [15] summarised evidence from 42 human clinical trials suggesting that increases in the genera of Bifidobacterium, Bacteroides, Faecalibacterium, Akkermancia Mucinophilia and Roseburia are negatively associated (beneficial), whereas the genera of Ruminococcus, Fusobacterium and Blautia are positively associated (detrimental) with severity of T2D. The above statements are supported by our findings, which show that over a 6-month period the beneficial bacteria such as Bifidobacteriaceae, Lactobacillaceae, and Bacteroidaceae families decline in the control arm (SU-only) but are promoted in SU+OZ101 treated arms (Fig 4a-c). In contrast, detrimental bacteria such as Streptococcaceae increased in control arm but decrease in patients taking OZ101 (Fig 4d). The latter also suggest that 6 months SU-only therapy promotes bacterial families known to be opportunistic. Strikingly, we also found that higher doses of OZ101 prebiotic also promotes bacteria families such as Lachnospiraceae, Ruminococcaceae and Erysipelotrichaceae, which have been previously shown to correlate with the severity of T2D (detrimental).

In this hypothesis generating, open-label and dose-range finding pilot study, adjunctive intake of 13.5 g/d OZ101, an oligofructose prebiotic, for 6 months safely improved HOMA-B (a marker of beta-cell function) whilst reducing further loss of glycaemic control in patients with T2D on long-term SU therapy. These findings may relate to improvement of gut microbiome dysbiosis at optimal OZ101 dose associated with both the diabetes per se, and the long-term SU use. The higher doses of OZ101 showed no significant effects on glycaemic control. Our preliminary observations suggest that higher doses of OZ101 increases the levels of bacteria shown to be positively correlated with severity of T2D (detrimental), which may counteract the increased beneficial bacteria in these patients, resulting in overall ineffective glycaemic control. To the best of our knowledge, this is the first clinical study that utilized high doses of oligofructose prebiotic above 15 g/d. Nevertheless, these preliminary findings suggest optimal dosage for OZ101 at 13.5 g/d. Our findings have potentially important clinical and public health implications. Although newer therapies are increasingly being used in the developed world, they are expensive and usage is more limited elsewhere. Sulphonyureas remain one of the two most widely prescribed drug classes internationally, but suffer from loss of efficacy over time. The use of a cheap adjunct therapy to prolong their useful life in individual patients whilst potentially improving the natural history of the disease has significant positive implications. Future studies with good statistical power and double-blind design aimed at confirming our findings and providing a better understanding of gut microbiome dynamics before and after adjunctive intake of OZ101 are now required. These will provide further insights into the mechanism by which treatment with optimal doses of OZ101 may reduce the rate of glycaemic deterioration and progressive beta-cell failure commonly observed in SU-treated patients.

Data Availability: The data underlying the findings of this manuscripts will be deposited in an appropriate publicly available data repository.

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this article. The authors NNG, KHSK, WLF, MA, JDH, CRK, TRH, AHB, SKP are shareholders at OzStar Therapeutics Pty Ltd.

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