

RESEARCH: EPIDEMIOLOGY

Effects of prebiotics on postprandial GLP-1, GLP-2 and glucose regulation in patients with type 2 diabetes: A randomised, double-blind, placebo-controlled crossover trial

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Abstract

Aims: We aimed to investigate the effect of prebiotic inulin-type fructans (ITF) versus a control supplement on postprandial levels of glucagon-like peptide-1 and -2 (GLP-1 and -2), glucose and insulin in people with type 2 diabetes.

Methods: Adult men and women with type 2 diabetes were randomised in a double-blind, placebo-controlled crossover study. The study participants received 16 g/d ITF and 16 g/d control supplement (maltodextrin) for 6 weeks each in two phases separated by a 4-week washout. A standardised mixed-meal test was performed before and after each intake period. The primary end point was changes in the GLP-1 response, and secondary end points were GLP-2, glucose and insulin responses. Data were analysed using mixed-model analysis.

Results: A total of 29 participants were included in the study. Differences between and within the two treatments in estimated area under the curves were not significant. Yet, the predicted means for meal-induced GLP-1 response in plasma showed a 4.8% decline after the prebiotic treatment and an 8.6% increase after the control treatment (difference in changes between the treatments, $p < 0.001$). Fasting or postprandial glucose, insulin or GLP-2 levels were not changed.

Conclusions: Our findings do not support that ITF improve incretin responses or glucose regulations in this population.

Clinicaltrials.gov (NCT02569684).

KEYWORDS

GLP-1, GLP-2, incretin effect, prebiotics, standardised mixed meal, type 2 diabetes

1 | INTRODUCTION

Lifestyle changes, including dietary modifications such as increased consumption of fibres, are fundamental approaches in both preventing and managing type 2 diabetes (T2D).¹ In recent years, supplementation with prebiotic fibres has been investigated as treatment strategy in obesity and metabolic disturbances.^{2–6} Prebiotic fibres escape digestion in the small intestine and are fermented in the colon by gut bacteria with presumed health-promoting properties, thereby nourishing their growth and activity.⁷ The short-chain fatty acids (SCFA) produced in the fermentation have been identified as signalling molecules that may bind to G-protein-coupled receptors (GPRs) and cause various effects depending on the tissues affected.⁸ In enteroendocrine L-cells, the SCFAs have been reported to increase the release of glucagon-like peptide-1 and -2 (GLP-1 and -2) in response to feeding, potentially improving the metabolic regulation in T2D, as well as preserving the intestinal integrity.⁸

Studies of the gut bacteria have revealed a moderately altered gut homeostasis in individuals with overweight and T2D,^{9,10} and this microbial divergence is suspected to contribute to the development of low-grade inflammation in several tissues and possibly to the onset of T2D.⁸

The inulin-type fructans (ITF) and galactans are the most extensively documented prebiotics to beneficially impact human health.⁷ Randomised controlled trials report positive effects of ITF on GLP-1 response¹¹ and glucometabolic parameters^{11–13} in populations without diabetes. Although increased GLP-1 response and improved glucose regulation could benefit individuals with T2D in particular, studies of the potential role of prebiotic fibres in this population are scarce. Previously published results from this cohort showed increased concentrations of faecal bifidobacteria and SCFA after treatment with ITF, significantly different from the control treatment.¹⁴

The primary aim of the present trial was to investigate the effect of a prebiotic supplement on GLP-1 responses to a standardised mixed meal in participants with T2D. Secondary aims were to evaluate the effects on the responses of blood glucose, insulin and GLP-2.

2 | METHODS

The trial had a randomised, double-blind, placebo-controlled crossover design. We chose a crossover approach due to large inter-individual variability in microbial

Novelty Statement

- Type 2 diabetes is associated with an imbalance in gut microbiota. Prebiotic fibres have shown beneficial effects on the gut bacteria and glucometabolic parameters in individuals without diabetes.
- Little is known about the effect of prebiotic fibres on response of glucagon-like peptide-1 (GLP-1), blood glucose and insulin in type 2 diabetes.
- In the present study, we found no evidence to support that the prebiotic inulin-type fructans positively affect GLP-1 response or glycaemic regulation in adults with type 2 diabetes.
- Intervention of longer duration may be necessary for changes in the gut bacteria to take hold.

response to dietary interventions, allowing each participant to serve as their own control. Participants were recruited from general practices and the outpatient diabetes clinic at Oslo University Hospital and by advertisements in social media, posters in the hospital lobby and pharmacies.

2.1 | Participants

Eligibility for participation was determined at a screening visit at a minimum of 4 weeks prior to enrolment. Criteria for inclusion and exclusion were described in a previous publication,¹⁴ but briefly eligible participants were adult men and women previously diagnosed with T2D and a BMI ≤ 40 kg/m², HbA_{1c} <86 mmol/mol (10.0%) that were not treated with insulin or GLP-1 analogues.

We screened 131 individuals for eligibility and randomly allocated 35 of these to start with either prebiotics or placebo (Figure S1). Long distance from home to the study centre was the main reason for exclusion. A total of 29 participants were included in the analysis for glucose and insulin. Analyses for GLP-1 and -2 were performed only for the 25 participants that attended all four visits. Four participants dropped out before study start, and six participants dropped out during the study. Three participants withdrew from the study by choice, reporting personal reasons. Three participants were excluded because they had to start with antibiotics for minor infections, one was excluded because of use of a probiotic supplement, and three dropouts were diagnosed with serious illness and were unable to continue.

2.2 | Study design

The trial was conducted between February 2016 and December 2017 at the Diabetes Research Laboratory, Oslo University Hospital, Aker and was approved by the Regional Ethics Committee for Medical and Health Research South East (REK 2014/1180). Written informed consent was obtained from all participants. The study was performed in accordance with the 1964 Declaration of Helsinki and later amendments.

The intervention consisted of 16 g per day of ITF (a 50/50 mixture of shorter chain oligofructose and inulin; Orafiti® Synergy1, Beneo GmbH, Germany) and a control supplement (maltodextrin 16 g per day). The study participants received the two supplements for 6 weeks each in two phases separated by a 4 weeks washout. The supplements were similar in appearance and taste and were provided in unlabelled and identical opaque sachets of 8 g. To allow for adaptation, the participants were instructed to consume one sachet per day the first week and two sachets per day the remaining 5 weeks. The powdered supplements were mixed into food or drinks by participants' choice and ingested whenever convenient.

For assessment of compliance, the participants were asked to return unused sachets. Diabetes medication was discontinued 2 days in advance of the visits, and the participants were instructed to avoid strenuous exercise 1 day prior to testing. All participants were told to maintain habitual lifestyle during the study.

2.3 | Sample size

Because of lack of previous data for sample size calculation, we based our estimates on changes in area under the curves (AUCs) for GLP-1 response after a pharmaceutical intervention where mean (two-sided 95% CI) difference between treatment and placebo was 2.34 (1.32, 3.35) pmol/L*min.¹⁵ A within SD of 3.78 was assumed, and this indicated a minimum sample size of 23 individuals to achieve 80% power at $\alpha = 0.05$. To account for dropouts and a possibly lower treatment effect due to differences in intervention and design we added 12 people, amounting to a total of 35 participants required for randomisation.

2.4 | Randomisation and blinding

Randomisation lists were generated using a randomisation command for two-by-two crossover studies in Stata version 14 software. Randomisation and product distribution

were performed by staff members otherwise not involved in the trial conduct. All participants and clinical investigators were blind to treatment allocation.

2.5 | Outcomes and data collection

The participants visited the hospital before and after both intervention periods. At each of the four visits, blood levels of GLP-1, GLP-2, glucose and insulin were assessed during a standardised mixed-meal test. Before the first intervention period, the participants answered a food frequency questionnaire (FFQ) for the assessment of their habitual diet. The participants reported changes in gastrointestinal symptoms after both intervention periods.

2.5.1 | Anthropometric measurements

Height was measured at the screening visit with a standard altimeter. A body composition analyser (Tanita BC-418MA Segmental Body Composition Analyzer) was used for measuring weight and bioimpedance before and after the two intervention periods. The participants were measured with bare feet and wearing light clothing.

2.5.2 | Blood sample collection and biochemical markers

After an overnight fast, the participants arrived in the morning at the research laboratory for a standardised mixed-meal test. Blood samples for glucose, insulin, GLP-1 and GLP-2 measurements were collected immediately prior to and 15, 30, 45, 60, 90, 120, 150 and 180 min after meal initiation. The meal consisted of two nutritional drinks (200 ml Fresubin 2 kcal Drink vanilla and 100 ml Fresubin Jucy Drink apple) containing 550 kcal, 78.5 g of carbohydrate, 24 g of protein and 15.6 g of fat. The drinks were consumed within 12 min.

Whole-blood glucose was measured immediately by a glucose oxidase method (YSI 2300; Yellow Springs Instruments), and plasma glucose concentrations were calculated (whole-blood glucose \times 1.119). Blood for insulin analysis was sampled in tubes without anticoagulant. Insulin was measured at the Hormone Laboratory, Oslo University Hospital, using Modular Analytics E170 (Roche, Switzerland). The minimum detectable concentration of the assay is 1.39 pmol/L, and inter-assay CV is $\leq 4\%$.

Blood for GLP-1 and -2 analyses was collected in EDTA tubes, to which were added 40 μ l of dipeptidyl peptidase-4 (DPP-4) inhibitors (Merck Millipore, Germany) and 40 μ l

of protease inhibitor (Pefabloc® SC, Merck Millipore, Germany). Plasma was separated by centrifugation at 3500× g at 4°C for 10 min and aliquots stored at −80°C in biobank for later analysis. GLP-1 and -2 were measured at Department of Biomedical Sciences, University of Copenhagen, Denmark. All samples were extracted in a final concentration of 70% (GLP-1) or 75% (GLP-2) ethanol before measurements. Total GLP-1 was measured as described by Orskov et al.¹⁶ using a radioimmunoassay (antibody code no 89390) specific for the C-terminal of the GLP-1 molecule and reacting equally with intact GLP-1 and the primary (N-terminally truncated) metabolite. Intact GLP-2 was measured using a radioimmunoassay originally described by Hartmann et al.¹⁷ The antiserum (code no. 92160) is directed against the N-terminus of GLP-2, and therefore, measures only fully processed, active GLP-2 of intestinal origin.

Sensitivity for both assays was below 1 pmol/l, and intra-assay coefficient of variation <10%.

2.5.3 | Assessment of diet

The FFQ used was a validated, self-administered questionnaire assessing the total diet.¹⁸ Participants completed the questionnaires based on eating habits during the last 6 weeks.

2.6 | Statistical analysis

For each of the four parameters of interest (glucose, insulin, GLP-1 and GLP-2), trajectories across the 9 measurement points were averaged over individuals at baseline and at each of the four visits for both the active and the control treatment. These empirical curves guided the choice of suitable functional forms for describing the mean concentration over the time span of 180 min from administering the mixed meal. The curves were also used for indications of differences between baseline and the follow-up measurements and differences between the two treatments. The curves suggested symmetric and asymmetric shapes and were consequently modelled by combinations of Time, Time² and log (Time).

Correlation induced by repeated measurements taken on the same individual was accounted for by applying mixed-effect regression models. Besides the time variables, the models included 'Day' (baseline/6 weeks follow-up), 'Treatment' (control/prebiotics), 'Period' (first/second) and 'Order of treatment' and were adjusted for age and sex. For normalisation GLP-1, GLP-2 and insulin were log-transformed, whereas glucose was fitted on the original scale. Finally, we checked the models for goodness of fit

by residual- and qq-plots. Calculation of AUC was done by numerical integration, and their uncertainties were assessed by bootstrapping.¹⁹

A potential correlation between GLP-1 and the associated microbial data¹⁴ was assessed by considering the residuals from the mixed model, constrained to the data from the control arm of the trial. The model was adjusted for age, gender and time variables and used 'Subject' as a random effect with 'Period' and 'Day' as random coefficients. All analyses were done using R ver.3.6.1. Figures showing empirical means of glucose, insulin, GLP-1 and GLP-2 at the four visits are included in Supporting information (Figure S2 and S3).

3 | RESULTS

3.1 | Participant characteristics

The mean (±SD) age of the participants was 61.5 ± 11.7 years, 12 (41%) were women, and they had a diabetes duration of 5.1 ± 4.4 years (Table 1). The baseline characteristics did not differ between the 25 participants that were included in the incretin analyses and the total study population. Compliance to the fibre supplementation was high with only mean (range) 3.3% (0%–20.8%) of

TABLE 1 Participant characteristics at baseline^a

Variable	n = 25 ^b	n = 29 ^c
Women	10 (40.0)	12 (41.4)
Age (years)	63.1 ± 11.5	61.5 ± 11.7
Fasting glucose (mmol/L)	8.7 ± 2.4	8.8 ± 2.4
BMI (kg/m ²)	29.1 ± 4.7	28.9 ± 4.5
HbA _{1c} (mmol/mol)	52 ± 11	52 ± 11
HbA _{1c} (%)	(6.9 ± 1.0)	(6.9 ± 1.0)
Dietary fibre (g/day)	32.2 ± 10.3	31.5 ± 10.2
Diastolic blood pressure (mmHg)	137.8 ± 18.2	136.3 ± 17.9
Systolic blood pressure (mmHg)	85.7 ± 10.1	85.6 ± 9.5
Diabetes duration (years)	4.7 ± 4.4	5.1 ± 4.4
Diabetes treatment		
Diet	8 (32.0)	8 (27.6)
Metformin	17 (68.0)	21 (72.4)
SGLT2 inhibitors	2 (8.0)	4 (13.8)
DPP-4 inhibitors	5 (20.0)	7 (24.1)
Sulfonylureas	1 (4.0)	1 (3.4)

^aData are mean ± SD or n (%); SGLT2, sodium-glucose cotransporter-2; DPP-4, dipeptidyl peptidase-4.

^bAnalysed for glucagon-like peptide-1 (GLP-1) and GLP-2.

^cAnalysed for glucose and insulin.

the prebiotic sachets and 4.3% (0%–22.1%) of the control sachets returned.

3.2 | GLP-1, GLP-2, glucose and insulin

The differences in GLP-1 response between and within the two treatments in estimated AUCs were not significant (Table 2). Yet, the model-based means showed a significantly different effect of the two treatments regarding changes in GLP-1 excursions ($p < 0.001$) (Table S1, Figure 1a–d). After prebiotics, the response decreased by 4.8% (log (GLP-1) estimated mean (SE) 0.049 (0.019) pmol/L, $p < 0.008$), whereas it increased by 8.6% after the control treatment (estimated mean (SE) 0.082 (0.019) pmol/L, $p < 0.001$).

The GLP-2 response to the mixed meal did not show any significant differences between the two treatments, in neither the shape of the curve parameters nor the corresponding AUCs (Table 2, Table S1, Figure 1e–h).

Glucose and insulin responses to the mixed meal remained unchanged during the trial (Table 2, Table S1, Figure 2).

3.3 | Possible interactions

The majority of our participants used metformin (72.6%), and the dose was kept unchanged throughout the trial. We found no effect of metformin on GLP-1 response. Post hoc analysis showed no correlations between changes in GLP-1 responses and microbiota at any taxonomical level.

3.4 | Adverse effects of intervention

After 6 weeks of treatment with prebiotic fibres, 16 participants (64%) reported passage of gas and flatulence being

worse or much worse than before, whereas only 2 participants (4%) expressed the same complaints after treatment with the control supplement ($p < 0.001$). There were no adverse events and no significant changes in other gastrointestinal symptoms during the trial.

4 | DISCUSSION

In this randomised, placebo-controlled, double-blind crossover trial, 6 weeks of treatment with 16 g/d of ITF did not positively affect GLP-1, glucose, insulin or GLP-2 responses to a mixed meal in participants with T2D. On the contrary, we found that the GLP-1 response decreased significantly after the prebiotic treatment, whereas it increased significantly after the control treatment.

Few of the published trials of prebiotic effects have focused on effects on glycaemic control in T2D, and we were only able to identify one single trial investigating GLP-1 response in these participants. Our findings are in accordance with the results from Roshanravan et al. where the participants with T2D showed no significant difference in GLP-1 response or glycaemic regulation after 6 weeks of treatment with 10 g of inulin per day.²⁰ Luo et al. and Pedersen et al. did not find any changes in glycaemic regulation after supplementing either fructo-oligosaccharides²¹ or galacto-oligosaccharides²² in T2D. Others, however, report reduced fasting glucose in T2D after treatment with 10 g of ITF.^{23,24} These trials had intervention periods lasting 2 weeks longer than the present trial. It could be argued that the intervention period in the present trial should have exceeded 6 weeks. However, extending the intervention periods would also increase the possibility of inducing weight loss, an effect previously demonstrated after prebiotic treatment^{12,13} that could confound other outcome measures in our trial. In addition, healthy individuals given the exact same type

TABLE 2 Area under the curves (AUCs) (95% CI) for marginal response curves of prebiotics on blood levels of log(GLP-1), log(GLP-2), glucose and log(insulin) during the 0–180 minutes interval after a standardised mixed meal^a

	Control		Prebiotics	
	Baseline	6 weeks	Baseline	6 weeks
GLP-1 (pmol/L*min)	6997.8 (6592.0, 7441.3)	7595.8 (7167.4, 8085.4)	7180.0 (6736.9, 7693.9)	6979.3 (6552.8, 7415.9)
GLP-2 (pmol/L*min)	6924.0 (6023.9, 7998.8)	7015.0 (6155.2, 7870.7)	6924.0 (6023.9, 7998.8)	7015.0 (6155.2, 7870.7)
Glucose (mmol/L*min)	2436.1 (2287.9, 2585.9)	2436.1 (2287.9, 2585.9)	2436.1 (2287.9, 2585.9)	2436.1 (2287.9, 2585.9)
Insulin (pmol/L*min)	65140.5 (55615.7, 75491.1)	65140.5 (55615.7, 75491.1)	65140.5 (55615.7, 75491.1)	65140.5 (55615.7, 75491.1)

Abbreviations: CI, confidence interval; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2.

^aCalculation of AUC was done by numerical integration and their uncertainties were assessed by bootstrapping. Effects of age, sex and period were integrated out of the areas. Equal values in various combinations of treatment and time reflects that no differences were detected in the modelling.

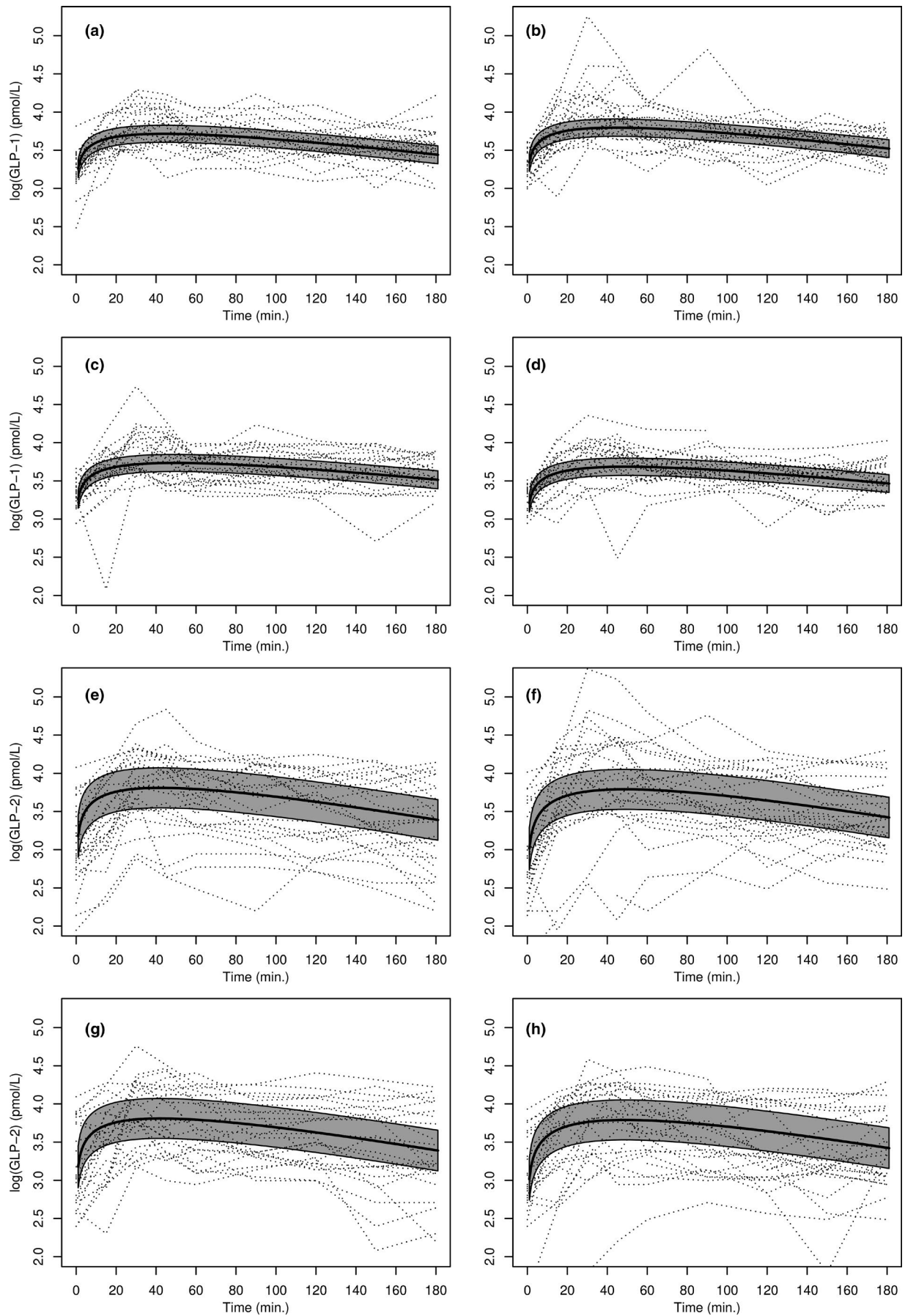


FIGURE 1 Predicted means with 95% confidence bands for the plasma concentrations of active GLP-1 (a, b, c, d) and active GLP-2 (e, f, g, h) in response to a standardised mixed meal before (baseline) and after (6 weeks) treatment with a control supplement (a, b, e, f) or prebiotics (c, d, g, h). Dotted lines are the observed individual trajectories. GLP-1 and 2, glucagon-like peptide-1 and 2

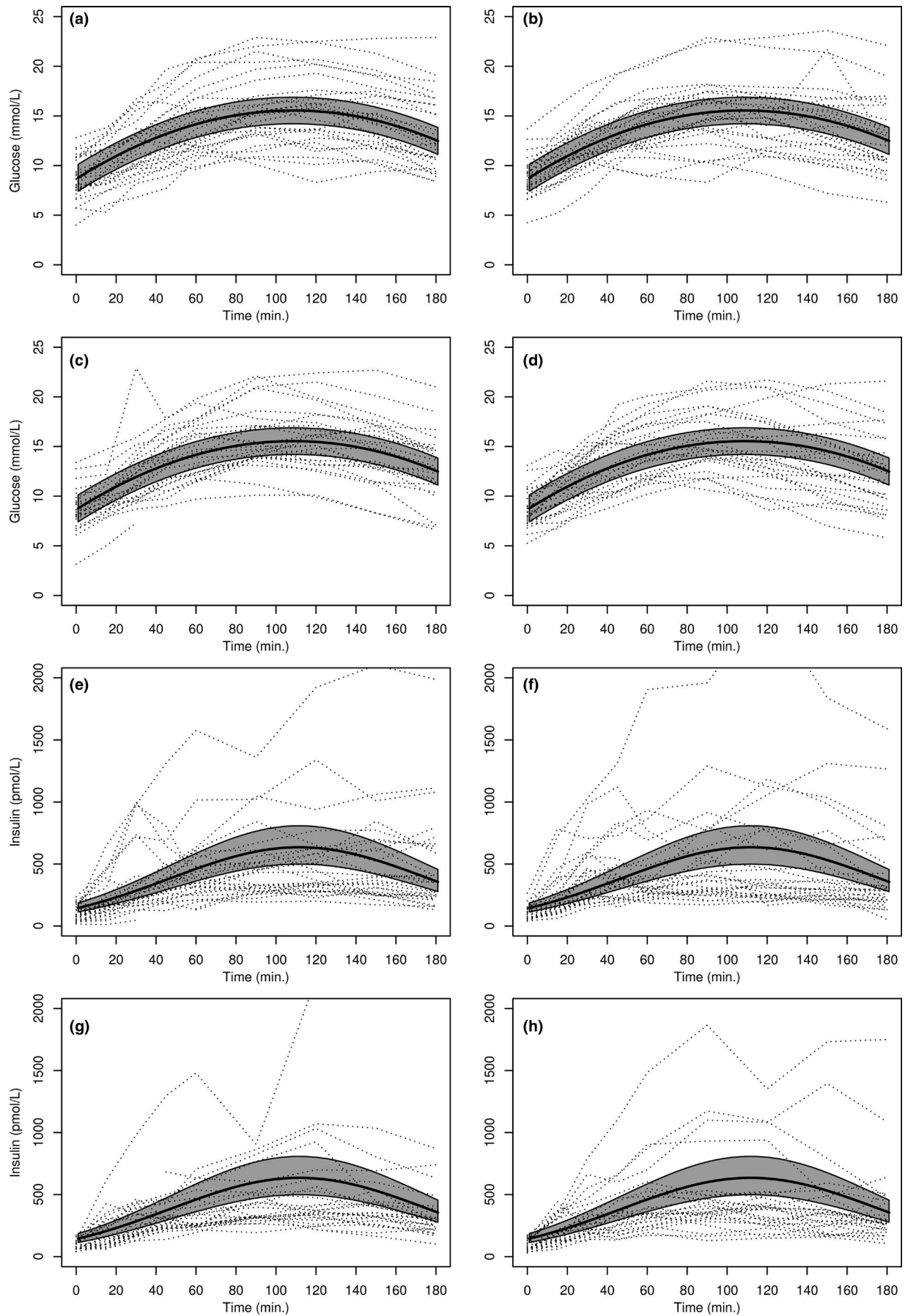


FIGURE 2 Predicted means with 95% confidence bands for the plasma concentrations of glucose (a, b, c, d) and serum concentrations of insulin (e, f, g, h) in response to a standardised mixed meal before (baseline) and after (6 weeks) treatment with a control supplement (a, b, e, f) or prebiotics (c, d, g, h). Dotted lines are the observed individual trajectories

and dose of ITF for only 2 weeks had increased GLP-1 response and reduced excursions of blood glucose in a study by Cani et al.¹¹ The dose of 16 g of ITF was decided after weighing the amounts of prebiotics sufficient to induce positive and clinically significant changes in gut microbiota and GLP-1 responses against doses low enough to avoid adverse side effects and minimise gastrointestinal discomfort.

The hormone GLP-2 maintains the intestinal integrity.⁸ The present trial appears to be the very first measuring GLP-2 response after the treatment with ITF in T2D. Russo et al., however, conducted a similar crossover study in healthy individuals. They measured GLP-2 response to a standard meal after a 5-week treatment with 11 g of inulin per day.²⁵ This induced a slight increase in fasting GLP-2, but no changes in postprandial GLP-2 response. In healthy participants, however, consumption of bread enriched with resistant starch for 3 days improved both insulin sensitivity and GLP-1 and -2 responses.²⁶

Our previously published results from this cohort showed increased concentrations of faecal bifidobacteria and SCFA after the prebiotic treatment, significantly different from the control treatment.¹⁴ Both human and murine trials report a potential for bifidobacteria in preventing endotoxemia and improving glucose regulation in T2D.^{27,28} In the present paper, we attempted to link the effect of prebiotics not only to metabolic outcomes but also to the changes in the gut microbiota. The increase in bifidobacteria induced by the prebiotics, however, did not have further beneficial impact on regulation of GLP-1, GLP-2 or blood glucose in our participants. The post hoc analysis also dismissed any correlations between changes in GLP-1 responses and gut microbiota.

It could be speculated that the positive changes in gut microbiota were too weak to enhance the release of GLP-1 and -2. An imbalanced gut microbiota such as reported in T2D may also warrant intervention of longer duration for the bacteria to establish necessary cross-feeding arrangements beneficial to the host. Similar to our study, others have demonstrated the positive effects on gut microbiota after supplementing prebiotics to overweight and prediabetic individuals, with no further implications on fasting GLP-1 nor glucose regulation.^{29,30} Pedersen et al. also analysed gut microbiota in addition to glycaemic regulation after supplementing galactooligosaccharides to participants with T2D. In contrast to our investigations, they found no significant microbial changes.²² The authors suggested failure to account for use of metformin and note the low dose of 5.5 g per day as possible explanations.

To our surprise there was an increase in GLP-1 response after the control treatment of 16 g of maltodextrin

per day. Maltodextrin is a readily digested and absorbed non-fermentable carbohydrate that is commonly used as placebo in trials like this. The carbohydrate content given in this trial was comparable with a small tablespoon of sucrose per day for 6 weeks, and the supplements were not taken the morning of the visits. We, therefore, believe that this is an arbitrary finding without clinical significance.

The strengths of our study include the randomised double-blind crossover design, excellent compliance, no dropouts due to the intervention, and a careful consideration of medication previously identified as possible confounders.^{9,31} Diabetes medication was kept unchanged during the trial except for the 48-hour cessation prior to visits, and no effect of metformin on GLP-1 response was found. Furthermore, only DPP-4 inhibitors with short terminal half-life were among the diabetes medications used in this trial. The gut microbiota responds to dietary changes within few days and normalises just as rapidly when the intervention discontinues.³² We, thus, regard a washout period of 4 weeks ample time to minimise the risk of carry-over effects.

A limitation to this study was a relatively large dropout rate of 10/35. Increased risk of participants dropping out is a known drawback with crossover studies.³³ Dropouts may induce bias and threaten the generalisability of the results. Because of the reasons for attrition, and data mainly being missing at random, we do not believe that the dropout rate has compromised the validity of the study. Another limitation to the study was failure to analyse hormones in the blood sampled from the four participants that did not attend all four visits. Still, according to the power calculation, we had sufficient participants to test our primary hypothesis. We also note that the intake of dietary fibre assessed with FFQ at the first visit (baseline) exceeded the inclusion criteria for some of the participants, as the evaluation of fibre intake at the screening was based on a simplified approach. Still, no significant correlation was found between reported fibre intake and changes in gut microbiota.¹⁴ Furthermore, as the study was a crossover trial, and the participants served as their own control, the influence of confounding covariates is expected to be minor. Like all dietary assessment methods FFQs are known to be confounded by both over- and under-reporting, and we cannot exclude a reporter bias because of the participants' awareness of the nature of this study.

5 | CONCLUSIONS

Supplementing 16 g of prebiotics per day for 6 weeks in people with T2D did not positively affect the response of GLP-1, GLP-2, glucose nor insulin to a standardised mixed meal. Our findings do not support a potential of ITF in

the regulation of GLP-1, GLP-2, glucose or insulin in this population.

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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