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Research Article

The Benefits and Risks of Probiotic, Prebiotic and Symbiotic interventions in the Care of patients with Diabetes Mellitus

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Abstract

Introduction: Probiotics, prebiotics and synbiotics are thought to affect the pathophysiology of diabetes mellitus including gut dysbiosis, intestinal barrier permeability and modulator of gut-brain axis and oxidative stress. This systematic review examined if their interventions resulted in improved clinical outcomes and were safe to administer.

Methods: An electronic search was conducted in August 2020 of CINAHL, EMBASE, MEDLINE, and PUBMED databases as well as using Google Scholar using keyword searches combined in a formal search strategy. The studies extracted were then filtered through an inclusion and exclusion criteria and assessed for risk of bias.

Results: Twenty-four studies met the inclusion criteria, with 20 studies involving participants with type 2 diabetes, 1 study a mixed cohort of type 1 and 2, and 3 involving prediabetes participants. Meta-analysis was not appropriate due to the heterogeneity in populations, methods and presented results. One trial was limited due to unclear risk of bias and was excluded. Four key themes were identified across the studies: improvements to glycaemic control; improvements in oxidative stress, inflammation and gut permeability; lipid profile, anthropometric parameters and blood pressure; and adverse events and tolerability.

Conclusions: Probiotics improved glycaemic control, oxidative stress, inflammation and gut permeability and lipid profile in T2DM participants. There was no evidence of improvements to T1DM due to lack of studies and insufficient studies on pre-diabetes. Synbiotics are also promising but prebiotics have insufficient evidence.

Introduction

The pathophysiology of diabetes mellitus (DM) is not homogeneous: Type 1 (T1DM) occurs in lean as well as obese people (Thomas, Jones, Weedon et al. 2018) and the progression of hyperglycaemia in T2DM varies from patient to patient (Faerch, Hulman and Solomon 2016). Moreover, though patients are surviving longer with the disease (Nishimura, LaPorte, Dorman et al. 2001), studies suggest that tight control of blood glucose, the cornerstone of treatment interventions, may not prevent macrovascular complications (Rodriguez-Gutierrez and Montori 2016), or microvascular ones (Boussageon, Pouchain, Renard 2017). The problems with clinical classification and uncertainty over efficacy of tight glycaemic control indicate that a way for more individualised treatments is required.

Gastrointestinal (GI) tract disorders are associated with diabetes and its complications including disturbed intestinal motility, secretion and absorption, diabetic gastroparesis and increased pathogens such as Candida (Wolosin and Edelman 2000). More recent studies on the gut microbiota have led to theories linking the pathophysiology of diabetes with imbalances or dysbiosis of the microbiota (Pussinen, Havulinna, Lehto et al. 2011).

Studies on the bacteria in the GI tract have shown the importance of microbes in producing energy for its human host (Wong, de Souza, Kendall et al. (2006), which may play a part in obesity (Flint, Scott, Duncan et al. 2012) and the development of T2DM. The disturbed microbiota, or dysbiosis, is also linked with development of T1DM with Paun, Yau and Danska (2017) linking the series of changes in childhood microbiota with the first measurements of autoantibodies associated with the disease. A second theory involves microbiota associated damage to the intestinal barrier (Delzenne and Cani 2011) leading to release of pathogens and antigens into systemic circulation resulting in inflammation. A

third theory concerning microbiome-triggered oxidative stress provides a route for the development of diabetes (Luca, Di Mauro, Di Mauro et al 2019). A fourth theory concerns the gut-brain-axis and the role of bacteria in stimulating release of hormones involved in satiety and insulin secretion (Xu, Zhou & Zhu 2017).

Studies have also found that individuals have widely different glycaemic responses to the same food consumed correlating with their gut microbes (Zeevi, Korem, Zmora et al. 2015). This could point the way to more individualised patient care and more effective, targeted, diet advice for diabetes.

Physiology of gut and microbiota

Throughout the GI tract, there are around 100 trillion microorganisms of at least 1,000 different species of known bacteria weighing up to 2kg (Flint, Scott, Duncan et al. 2012). The microorganisms vary in density and richness along the length of the GI tract, shaped at different sites with the mouth, stomach, small intestine and colon providing vastly different microenvironments due to changes in pH, transit time, and occurrence of enzymes (Sekirov, Russell, Antunes et al. 2010).

The functions of the microbiota include assisting with digestion of carbohydrates, fat and proteins, producing key vitamins and metabolism of dietary fibre into short chain fatty acids (SCFAs) mainly acetate, propionate and butyrate (Knight, Bayram-Weston and Nigram 2019). Butyrate repairs and enhances the intestinal barrier function but also has a paradoxical role in glucose, lipid and energy metabolism (Liu, Wang, He et al. 2018). Propionate regulates hepatic gluconeogenesis and satiety while acetate is involved in cholesterol metabolism and lipogenesis at peripheral tissue sites (Valdes, Walter, Segal, et al. 2018).

Influences on microbiota

Numerous factors are known to affect the form and function of the microbiota. While some of the microbiota appears to be inheritable (Goodrich, Davenport, Beaumont et al. 2016), environmental factors play a larger part in determining its composition especially diet, drugs, type of delivery at birth and method of infant feeding (Rothschild, Weissbrod, Barkan et al. 2018).

Drug interventions can affect changes to the microbiota. Treatment with systemic antibiotics results in a decrease in microbial diversity (Langdon, Crook and Dantas, 2016). Commonly used non-antibiotic drugs also change the microbiota including Metformin Hydrochloride **(**Wu, Esteve, Tremaroli et al. 2017) and proton pump inhibitors (PPIs) (Weersma, Zhernakova and Fu, 2020). Air pollution also modifies the microbiota through particulate matter (PM) contamination of food and water and through inhalation (Salim, Kaplan and Madsen, 2014).

Theories of role of microbiota in pathophysiology of DM

Dysbiosis and increased energy harvest

The role of SCFAs in energy balance and as excess source of energy is suggested for development of T2DM. Together with energy harvest from fermentation of dietary fibres, Fluitman, De Clercq, Keijser et al. (2017) describe several other roles for SCFAs in energy balance, including influences on glucose and lipid metabolism and regulation of fatty acid oxidation. They found conflicting evidence for studies examining the role of SCFAs in energy balance with some associating increased levels of SCFAs in obese subjects compared to lean ones as well as studies linking administration of SCFAs to weight loss. The question of whether dysbiosis is a direct cause of any metabolism-related disorder or a consequence of the change in the host's diet remains uncertain (Carding, Verbeke, Vipond et al 2015). Den Besten, van Eunen, Groen et al. (2013) found that the process of producing SCFAs necessitates the microbiota to work in cross collaboration in order to produce desired quantities and remove unwanted by-products. They said that the supply rates of SCFAs in humans remain unknown as well as the information on the carbohydrates and microbiota needed to influence mass and composition of SCFAs. The research is hampered by a lack of human data on gut concentrations of SCFAs rather than faecal ones.

Role of microbiota in impaired intestinal barrier

The theory of an impaired intestinal barrier is implicated in the development of T1DM. Vaarala, Atkinson and Neu (2008) in a theory of the 'Perfect Storm' reviewed studies of patients with increased intestinal permeability in subjects with the disease or at risk of developing it. They suggest an altered microbiota causes increased permeability which leads, via cytokine release or an autoimmune process, to pancreatic islet inflammation and beta cell destruction. Enteric pathogenic bacteria and lipopolysaccharides, a component of Gram-negative bacteria, are known to alter the tight junction (TJ) at the epithelium causing inflammation (Lee, Moon and Kim 2018).

The microbiota's role in intestinal inflammation is also suggested as a route to T2DM. Ding and Lund (2011) say a high-fat diet (HFD) interacts with bacteria in the microbiota to drive inflammation, obesity and insulin resistance with the site of the small intestine of particular importance. However, Thaiss, Levy, Grosheva et al. (2018), in studies of mice, found that hyperglycaemia caused impaired intestinal barrier and susceptibility to systemic spread of enteric pathogens independent of disruptions to the microbiota.

Research strongly suggests a role for microbiota in maintaining stable intestinal barrier but Thaiss et al.'s (2018) study on the pivotal role in hyperglycaemia in impaired barrier throws doubt on the efficacy of interventions of the microbiota on reducing permeability in the presence of hyperglycaemia.

Microbiota-Gut-Brain Axis

The microbiota is known to modulate the gut-brain axis (GBA), the bidirectional talk between the enteric nervous system and the brain, to influence endocrine and metabolic pathways (Carabotti, Scirocco, Maselli et al. 2015). SCFAs can stimulate the release of cells, neuropeptide YY (PYY) and glucagon-like peptide type 1 (GLP-1), signalling to the brain to induce feelings of satiety, inhibiting intestinal motility and improving glucose metabolism (Xu et al. 2017).

The crosstalk in the GBA includes intestinal glucose sensors in the gut signalling to the hypothalamus to control glucose entry to tissues which becomes disrupted in presence of GI tract inflammation (Bessac, Cani, Meunier et al. 2018). This suggests a role for the microbiota in modulating gut inflammation and the GBA.

Role of microbiota in oxidative Stress

The role of oxidative stress (OS), where reactive oxygen species (ROS) build up to harmful levels, overwhelming the body's supply of antioxidants and causing cellular damage has been established in pathophysiology of diabetes including promoting microvascular and cardiovascular complications (Giacco and Brownlee, 2010). The mitochondria is the major producer of ROS and aberrant production of ROS is modulated by the microbiota and its SCFAs (Luca et al 2019). SCFAs are also the main source of energy for colonocytes (Den Besten et al. 2013), colonic epithelial cells, which maintain anaerobic conditions in the gut lumen by rapidly metabolising oxygen (Litvak, Byndloss and Baumler, 2018). .

Evidence for microbiota targeted treatment of DM through probiotics and probiotics and relevance to global health

Randomized controlled trials (RCTs) into the role of biotics in improving glycaemic control have been inconsistent. Some trials report increased insulin sensitivity after probiotic intervention. Rajkumar, Kumar, Das et al. (2015) carried out an RCT over 6 weeks using 45 healthy volunteers split into 3 equal groups and administered a placebo, a probiotic and a joint probiotic/prebiotic. All groups sustained a decrease in serum insulin which was significantly lower in the probiotic and synbiotic group with the greatest effect seen in the synbiotic group. Gurung, Li, You et al. (2020) found evidence from animal studies for certain probiotics in improving glucose tolerance and insulin resistance and some evidence from studies linked to improved T2DM symptoms in humans.

Ho, Nicolucci, Virtanen et al. (2019) reviewed the effects of prebiotics on glycaemic control, intestinal permeability and gut microbiota of children with T1DM. No changes were observed in glycaemic control or adverse events, but modest improvements were observed in intestinal permeability and changes to microbiota. Importantly, the intervention group had increases in C-peptides suggesting an improvement in beta cell function which could lead to improved glycaemic control over a longer intervention period. Tenorio-Jimenez, Martinez-Ramirez, Gil et al. (2020) examined probiotics on metabolic syndrome from randomized controlled trials (RCTs). They found improvements in subjects with metabolic syndrome including glucose metabolism in some studies. Nikbakht, Khalesi, Singh et al. (2018) observed a borderline statistically significant affect. Ruan, Sun, He et al. (2015) found a modest improvement in glycaemic control. Some studies have questioned whether the use of probiotic supplements aids recovery of normal gut microbes (Suez, Zmora, Zilberman-Schapira, et al 2018) and it remains unclear if natural probiotics in food are superior to supplements.

Manipulation of the microbiota could pave the way for creating a personalised care plan to improve the effectiveness of diabetes management.

The aim of our research, therefore, was to examine the effectiveness and safety of probiotics, prebiotics and synbiotics in glycaemic control of patients with diabetes or prediabetes.

Methodology

Search Strategy

A three-step search strategy was applied in this review aimed at classifying all eligible published studies. First, CINAHL, Embase, Medline, PubMed databases, and Google Scholar were searched by one of the research team. An initial limited search was first undertaken to identify articles on the topic. The text words contained in the titles and abstracts of relevant articles, and the index terms used to describe the articles were then used to develop a full search strategy for the report. The search strategy, including all identified keywords and index terms, were adapted for each included information source. Search terms used included key words and medical subject headings (MeSH): 'Type 1 Diabetes Mellitus' OR 'Type 2 Diabetes Mellitus' AND 'Adult' AND 'Probiotics' OR 'Prebiotics' OR 'Synbiotics' AND 'HbA1c' OR 'Glycated haemoglobin' OR 'blood glucose'. The search was limited to studies involving humans and published in English between 2010 and 2020.

Second, a process of screening, supplementary search parameters were used to ensure relevance to the topic, duplicate articles and those not relevant to our MESH terms were removed. Following abstract review, studies were excluded if they were not primary research, unrelated to topic, excluded human participants, non-English language and did not have full text availability for the review.

Finally, the full text of selected citations was assessed in detail against the inclusion criteria by two independent reviewers. Reasons for exclusion of full text studies that did not meet the inclusion criteria was recorded and reported in the systematic review. Disagreements between the reviewers at each stage of the study selection process were all resolved through discussion, and by including a third reviewer if required. The results of the search were reported in full in the final systematic review and presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)

flow diagram (PRISMA, 2009). Of the 187 papers generated using the keywords, 24 papers were included for the final analysis.

Inclusion/exclusion criteria

The inclusion and exclusion criteria was developed using a PICO structure (population/patient, intervention, comparator, outcome). Trials were included if they recruited T1DM, T2DM or prediabetic participants who were over 18 years of age. Trials that included participants with other diseases who did not have DM were excluded if there was no separate analysis of the effect on DM participants. Interventions were included if they consisted of probiotics, prebiotics or a mixture of the two. Randomised control trials (RCTs) were selected. However, one case control trial was also included as it presented additional information that was unavailable in the RCTs. Outcomes relating to glycaemic control, inflammation, oxidation and endotoxaemia, anthropometric and lipid changes, and changes to the microbiota were included. Adverse effects were also included.

The database results were imported to Endnote software which removed duplicate results and enabled screening for studies of interest.

Full text articles were retrieved for quality assessment if they met the following criteria: randomized control trial (RCT), case-controlled trial (CCT), cohort trial and case studies. Multiple journal reports of the same trial were identified and linked together. Non-RCTs were included in the quantitative synthesis if they added information that was not covered by an RCT and could therefore provide insight otherwise overlooked. Some RCTs retrieved had not looked at glycaemic parameters but had looked at other important clinical outcomes such as anthropometric factors, lipid profiles, or oxidative stress and were included.

Data extraction and risk of bias

Data from eligible reports were extracted relating to the participant groups, length of trial, intervention, comparator, the outcome measured and trial design from each included trial. Where information was not provided in the study papers, further searches were made of trial protocol or any online supplementary papers.

The risk of bias was assessed using the Cochrane risk-of-bias tool for randomized trials (RoB2) (Sterne, Savovic, Page et al 2019) applying a series of signalling questions to the trial details. The risk of bias was assessed as low, high or unclear according to criteria described in RoB2.

Results

The database searches produced 179 articles in total and a further 8 from other sources. After the duplicates were removed, there were 67 articles remaining. Screening for irrelevance such as gestational DM, review articles and study protocols excluded a further 30 records. Full study details were obtained for the remaining 37 records with 5 journal articles linked to 2 trials. A case control study was discarded as it failed to add additional material covered by RCTs and others discarded because they were mixed cohorts of DM and other diseases. 24 studies were suitable for inclusion in the quantitative synthesis.

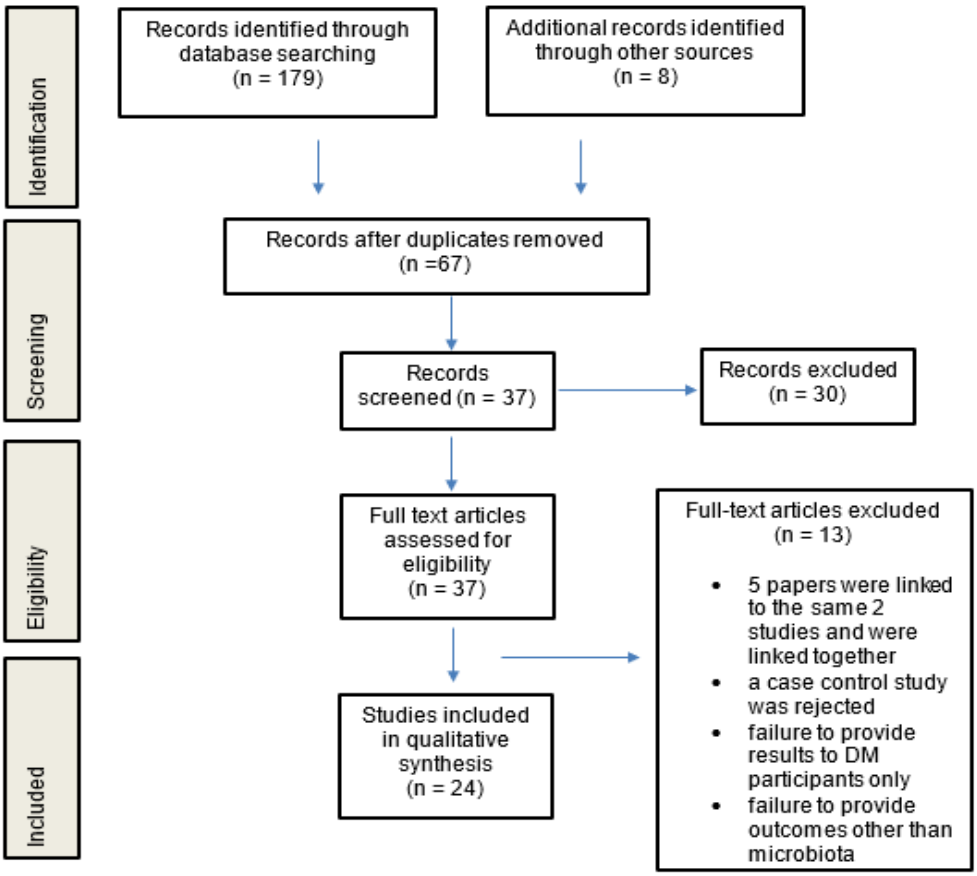

Figure 1: Prisma Flow Diagram indicating studies included

Analysis of included research papers

The characteristics of the 24 included trials are summarised in Table 1. One was a

Case-control study and the remaining 23 were RCTs. There was significant heterogeneity between the RCTs. The RCT intervention periods ranged from 4 weeks to 6 months and two studies had follow ups (4 and 5). One of the RCTs (15) was a crossover study with a wash-out period of 3 weeks. Sizes of intervention groups in RCTs ranged from 7 (study 24) to 48 (study 1). Characteristics of study populations and groups varied significantly between trials ranging from a mean age of 44 years (study 6) to 66 years (study 8, IG1), with similar unevenness in gender balance, glycaemic control and duration of disease.

Majority of the studies reported on interventions with T2DM. However, one study (21) included a small number of T1DM participants alongside T2DM, while 1 RCT reported newly diagnosed T2DM (18) as did the case control (3). Three studies reported only on prediabetic patients (22, 23 and 24).

Some RCTs excluded populations taking insulin as diabetic control while other included them. Several trials excluded participants who had some form of GI disorder (4, 6, 8, 9, 10, 11, 13, 14, 15, 18, 19, 20, 22, 23 and 24) and those who had taken antibiotics, and/or probiotics and/or prebiotics within a recent specified cut-off point (7, 8, 9, 10, 11, 12, 14, 16, 18, 19, 20, 21, 22, 23 and 24). Participants of studies 7 and 9 received therapeutic dietary and/or lifestyle advice alongside intervention, study 4 included vitamins and minerals alongside intervention. Study 20 involved participants with microalbuminuria and study 21 involved participants with chronic kidney disease (CKD). The doses varied considerably in strength, constituents and timing of consumption.

The risk of bias was assessed using the Cochrane risk-of-bias tool for randomized trials (RoB2) (Sterne et al 2019) applying a series of signalling questions to the trial details. Four studies had high risk in a single domain: study 8 was judged to have high risk of bias arising from randomisation process due to the placebo having a 'sweet taste' that could have distinguished it from intervention and study 15 was judged to have high risk of bias from measurement of outcome due to its trial design as a crossover study. However, it was retained as it was the only study that reported on uric acid levels. Studies 13 and 17 were judged to have high risk of bias due to missing data in some reported results. Where missing data was evident, no inclusion of significant results were included in the synthesis. However, study 17 reported no baseline or absolute changes for any reported areas of significance and was not included in any synthesis.

Study no., lead author year of publication, country of trial registration.	Population	Gender M/F	Length trial and type	Mean age and characteristics of Intervention (I) group(s) at baseline	Intervention including description, dose and timing where recorded	Other significant intervention
1. Firouzi et al 2017, Malaysia	101 T2DM, IG=48	54/47	12 weeks, RCT (parallel, 2 arms)	IG=53 years, HbA1c =7.58, BMI= 29.2, no insulin	Probiotic: 6 strains twice per day before or after food	
3. Greenway et al 2014, USA	1 T2DM	1/0	Case control	Case = 30 years, HbA1c=8.8, BMI=38.3	Cobiotic: inulin and blueberry antioxidant twice per day before meals	
4. Horvath et al 2019, Austria	26 T2DM, IG=12	19/07/2021	6 months RCT (parallel, 2 arms). Follow up at 12 months	IG=61 years, HbA1c= 8.0, BMI=33, excludes GI disorders	Synbiotic: Probiotic with 9 strains taken am. Prebiotic of GOS and FOS taken pm	Compounds of Mg, Mn, KCl and in intervention and placebo. Intervention also contained Ca, Zn compounds and vitamins B2 and D3.
5. Hsieh et al 2018, Taiwan	68 T2DM I1=22, I2=24	I1=12/10, I2 13/11	6 months RCT (parallel, 3 arms). Follow up at 9 months	IG1=52.3, IG2=53.9. HbA1c I1=7.9, HbA1c I2=8.07	Probiotic: IG1: live strain IG2:heat-killed strain	
6. Khalili et al 2019, Iran	40 T2DM IG=20	I=7/13	8 weeks RCT (parallel, 2 arms)	IG=44 years. HbA1c=7.3, BMI=29.5 No GI inflammation. No insulin	Probiotic: 1 strain taken daily with meal containing fats	
7. Kobyliak et al 2018, Ukraine	53 T2DM IG=31	Not stated	8 weeks RCT (parallel, 2 arms)	IG=52 years, HbA1c=8.4, BMI=34.7. Excludes antibiotics and pro/prebiotics with last 3 months	Probiotic: 14 live strains daily	Therapeutic diet advice

8. Mobini et al 2017, Sweden	44 T2DM IG1=15 IG2=14	35/11	12 weeks RCT (parallel, 3 arms)	IG1=66 years, HbA1C 7.8, BMI 30.6. IG2=64 years, HbA1c 8.1, BMI 32.3. All abdo obesity. All receiving insulin. Exclude inflammatory bowel disease, antibiotics within 4 weeks of trial, probiotics within 3 weeks of trial.	Probiotic: IG1 1 strain low dose. IG2 1 strain high dose. Control arm had 'sweet taste	
9. Palacios et al 2020, Australia	60 T2DM or prediabes IG=30	28/32	12 weeks RCT (parallel, 2 arms)	IG=61 years. HbA1c 6.1, BMI 35.5. DM or prediabetes diagnosed within previous 12 months, metformin or diet controlled only. No GI disorders, no antibotics or pro/prebiotics within previous 4 weeks	Probiotic: 8 strains, 2 capsules per day	Received lifestyle advice in both arms
10. Pedersen et al 2016, UK	29 T2DM IG=14	29/0	12 weeks, RCT (parallel, 2 arms)	IG=57 years. HbA1c 6.8, BMI 28. No GI disorders, no antibiotics in previous 3 months, no pre/probiotics in previous 2 weeks.	Prebiotic: GOS 5.5g per day	
11. Perraudeau et al 2020, , USA	58 T2DM IG1=21, IG2=21	22/36	78 days, RCT (parallel, 3 arms)	IG1=49 years, HbA1c=8.5, BMI 34.4. IG2=51 years, HbA1c=8.8, BMI =31.9. DM controlled by diet/exercise or metformin or with sulfonylurea. Excludes GI disease. Exclude use of antibiotic and probiotic, antifungal/antiparasitic/antiviral within previous 30 days	Probiotic: IG1 3 strains, IG2 5 strains. Probiotic strains anaerobic. Also includes inulin. 3 caps twice daily: breakfast and evening meal	
12. Razmpoosh et al 2019, Iran	60 T2DM IG=30	33/27	6 weeks, RCT (parallel, 2 arms)	IG=59 years, BMI=27.7, Exclude insulin, antibiotics and probiotics within previous 2 months.	Probiotic: 7 'live' strains and 100mg FOS, Mg Placebo: 100mg FOS, and Mg. 2 doses per day after lunch, after dinner	
13. Roshanravan et al 2017, Iran	59 T2DM IG1=15, IG2=15, IG3=14	22/37	45 days, RCT (parallel 4 arms)	IG1=46 years, BMI 29.8, IG2 = 51 years, BMI 30, IG3=47 years, BMI 30. Exclude insulin and GI disorders	IG1 Butyrate 6 caps per day IG2 Inulin 5g twice per day IG3 Butyrate + Inulin 6 caps + 2x5g	
14. Tonucci et al 2017, Brazil	45 T2DM IG=23	26/19	6 weeks, RCT (parallel, 2 arms)	I=52 years, HbA1c=6.07, BMI 27.5. Exclude insulin and GI disorders, antibiotics or probiotics in last 3 months. Exclusion of antibiotics/pre/probiotics at time of recruitment.	Probiotic: 2 live strains in fermented milk. Control group: fermented milk	

15. Asemi et al 2014, Iran	62 T2DM IG=31	19/43	6 weeks, RCT (cross over, 2 arms) with 3 weeks washout	IG and C mean age =53.1 years. BMI 29.6. Excludes insulin and short bowel syndrome	Synbiotic: 1 'viable and heat-resistant' strain and 0.36g inulin taken 3 times a day.	
16. Farrokhian et al 2019, Iran	60 T2DM IG=28	22/38	12 weeks, (parallel, 2 arms)	IG=64 years, BMI=32.3, with coronary heart disease. Excludes use of synbiotics/probiotics within past 3 months	Synbiotic: Probiotic 3 strains, prebiotic 0.8g inulin per day	
17. Mirmiranpour et al 2020, Iran	115 T2DM IG1=30, IG2=28, IG3=30	49/66	3 months RCT (parallel, 4 arms)	IG1=59.7 years, HbA1c 7.42, IG2=58.8 years, HbA1c 7.68, IG3=58.4 years, HbA1c 7.66. Excludes insulin	IG1 Probiotic: 1 strain. IG2: cinnamon IG3: Synbiotic – Probiotic plus cinnamon. Dose taken once daily with breakfast	
18. Sabico et al 2019, Saudi Arabia	61 T2DM IG=31	40/38 before dropout	6 months RCT (parallel, 2 arms)	IG= 48 years, BMI 29.4. Newly diagnosed, excludes poor glycaemic control and GI disorders, excludes insulin, prebiotics, probiotics and antibiotics	Probiotic: 8 strains, freeze dried twice per day before breakfast and before bed	
19. Tajadadi-Ebrahimi et al 2014, Iran	81 T2DM IG=27	15/66	8 weeks, RCT (parallel, 2 arms)	IG= 51.3 years, BMI 30.8. Excludes, insulin, inflammatory diseases, short bowel. No use of biotics for preceding 2 weeks	Synbiotic: probiotic and inulin in bread. Dose 40g bread three times per day	
20. Ebrahimi et al 2017,, Iran	70 T2D IG=35	42/28	9 weeks, RCT (parallel, 2 arms)	IG=59 years, HbA1c 7.44,BMI 27.3. DM>5 years with microalbuminuria. Excludes insulin therapy and GI disorders, use of synbiotics and antibiotics	Synbiotic: probiotic from 3 groups, + FOS	Interventions include 1mg Vit B, 0.5mg lactose, and Mg
21. Soleimani et al 2019, Iran	60 4 T1DM and 56 T2DM IG=30	22/18, RCT	12 weeks (parallel, 2 arms)	IG=63 years. BMI 26.4. Undergoing renal dialysis. Excludes use of probiotics/prebiotics in recent past.	Synbiotic: probiotic 3 strains + 0.8g per day inulin	
22. Canfora et al 2017, Netherlands	44 preDM IG=21	23/21, RCT	12 weeks (parallel, 2 arms)	IG=59 years. BMI 33.3. Overweight/obese, excludes GI disorders, use of antibiotics, prebiotics or probiotics within last 3 months	Prebiotic: GOS 5g 3 times per day with food	Supplements provided with yogurt drink

23. Kassaian et al 2020, Iran	85 pre DM IG1=27, IG2=30	38/47,	6 months RCT (parallel, 3 arms)	IG1=53 years, BMI 29.6. IG2=53 years, BMI 29.1. Exclude bowel disorders and use of antibiotics within last 3 months, exclude use of prebiotics and probiotics within last 3 months	IG1=Probiotic 4 strains IG2=Synbiotic, probiotic + inulin Dose diluted in water and taken with main meal daily	
24. Yang et al 2015, USA	13 preDM, IG=7 16 healthy controls	13/16,	8 weeks RCT (parallel, 2 arms)	IG=55 years, BMI 32.2. Excludes GI disorders, use of antibiotics within last 2 months.	IG = prebiotic XOS 2g per day	Intervention contained 20mg maltodextrin. Subjects asked to avoid other sources of XOS and probiotics

Table 1: Characteristics of studies included for systematic review and risk of bias

Discussion

Effect on glycaemic parameters

Seven studies (1, 5,6,8,11,14 and 18) on probiotics found evidence for improvement on glycaemic control, ranging from 6 weeks to 6 months (Table 2). Studies 1, 5 and 14 found improvements to HbA1C, the glycated haemoglobin which reflects average glucose concentrations in blood over an approximate 8-12 weeks. Study 14 was unusual as it resulted in improvements in HbA1c in a short trial of only 6 weeks though the significant value resulted from changes to placebo rather than baseline. Study 11 found that incremental glucose under the curve was also improved suggesting a postprandial effect. A review by Grom, Coutinho, Guimaraes et al (2020) found that postprandial glycaemia could be reduced by probiotics by inhibiting two enzymes in the small intestine thereby delaying the digestion of carbohydrates and slowing absorption of glucose.

Study no.	**Significant changes within group and compared to control arm to Glycaemic parameters reported**	**Significant changes within group and compared to control arm to anthropometric parameters and/or lipids and/or blood pressure reported**	**Significant adverse events reported**	**Significant changes within group and compared to control arm to biomarkers for oxidative stress, inflammation and gut permeability reported**	**Significant changes within group and compared to control arm to Microbiota reported**
1	HbA1c decreased by 0.14% (±0.62) between baseline and week 12. Fasting insulin decreased by 2.3 ±(6.8) µU/mL and 2.9 (±8.5) µU/mL between baseline and weeks 6 and weeks 12 respectively.	Systolic BP decreased by 8.1mmHg between baseline and week 12. Female waist circumference decreased by 2cm between baseline and week 12.	Minor gastric disturbance. Two events unlikely due to trial		Increase in species of Bifidobacterium and Lactobacillus

2					Gut diversity reduced in IG. Increase in abundance of Bifidobacteriaceae but other taxa decreased in abundance. α-diversity/Shannon Index decreased. Decrease in propionic acid.
3	Fasting blood sugar decreased	Weight decreased			
4		Hip circumference decreased by 1cm between baseline and month 6	Flatulence and diarrhoea	Serum zonulin reduced in IG by 0.05ng/ml between baseline and 3 months.	The probiotic strain was detected in samples at 6 months at end of trial but not at follow up at 12 months.
5	HbA1c decreased by 0.35% (±0.74)and 0.39% ±(0.8)in IG1 between baseline and month 3 and baseline and month 6 respectively	Cholesterol decreased by 4.45mg/dl between baseline and month 3 in IG1. Systolic BP and mean blood pressure decreased by 7.54mmHg and 4.63mmHg respectively between baseline and month 6 in IG2.		IL-1B decreased by 4.43pg/ml between baseline and month 6 in in IG2	Increase in *L. reuteri* in IG1. Increase in Bifidobacterium in IG2
6	Fasting blood glucose, fasting insulin and HOMA-IR decreased by 28.36(-45.39 to-11.31)mg/dl, 2.33 (-4.48 to-0.18) mU/ml and 29.72 (-45.62 to-13.82) respectively between baseline and week 8	Weight, waist circumference and BMI decreased by 1.2kg, 2.15cm and 0.485kg/m2 respectively between baseline and week 8.			
7		BMI, weight, and waist circumference decreased by 0.26kg/m2, 0.94kg and 0.75cm respectively between baseline and week 8	Diarrhoea, nausea and abdominal pain in 3 participants	Decreases of 7.95pg/ml in TNF-α, 5.44pg/ml in IL-1β, and 3.45pg/ml in IL6.	
8	Insulin sensitivity index (ISI) increased in IG2 by 0.4mU between baseline and week 12.	Weight and BMI increased by 0.9kg and 0.3kg/m2 in IG1between baseline and week 12.	Not significant compared to placebo		Increase in *L. reuteri* in IG1 and IG2 between baseline and week 12. No shift in gut diversity or overall composition
9			GI symptoms observed were not significant between groups		Increase in plasma butyrate concentrations between baseline and week 12.
10	Glucose effectiveness at zero insulin (GEZI) in decreased by 0.23/min	Body fat increased by 0.8% between baseline and week 12			Shannon indices reported increase in diversity and richness between baseline and week 12

11	Total glucose Area Under Curve (AUC) in IG2 and incremental glucose AUC in IG1 and IG2 decreased by 14.9mg/dL/180min, 3.69mg/dL/180min and 11.79mg/dL/180min respectively between baseline and week 12		GI symptoms –short lasting diarrhoea nausea, vomiting but not significant compared to placebo.		Detection of some probiotic strains at weeks 4 and 12. Increases in concentration of SCFA butyrate in IG1 and IG2 between baseline and week 12.
12	FPG decreased by 13.8mg/dL between baseline and week 6	Increase in HDL of 2.1mg/dl between baseline and 6 weeks			
13		Actual figures not reported in study.	One severe GI symptoms in IG3	Decreases in mean serum MDA of 1.41nmol/mL in IGI, 0.27nmol/mL in IG2 and 1.17nmol/nL in IG3. Decreases of hs-CRP of 1.35mg/L in IG1, 1.65mg/L in IG2 and 1.45mg/L in IG3.	Increase in *A. muciniphila* in IG1 and IG2 between baseline and day 45.
14	HbA1c decreased by 0.67% between baseline and week 6	Total cholesterol and LDL decreased by 0.15mmol/L and 0.2mmol/L respectively between baseline and week 6.		Actual figures not reported in study.	Increase in SCFA acetic acid in IG between baseline and week 6.
15	Serum insulin levels decreased by 1.75 µIU		No, but increase in serum uric acid levels in IG	Decreases in hs-CRP by 1058ng/mL and increase in GSH of 320µmol/L.	
16				Decrease in hs-CRP of 2,632ng/mL, increase in NO of 7. 6µmol/L, decrease in MDA of 0.6µmol/L.	
17	Missing data			Missing data	
18	HOMA-IR decreased by 3.2 and 3.4 between baseline and months 3 and 6 respectively. FPG decreased by 3.2mmol/L and 4.5mmolL between baseline and months 3 and 6 respectively.	Triglycerides decreased by 0.8mmol and 1.2mmol between baseline and months 3 and 6 respectively. Total cholesterol decreased by 1.1mmol/l between baseline and month 6. Total cholesterol/HDL ratio decreased by 1.1 between baseline and month 6. HDL increased by 1.1mmol/l and 1.3mmol/l respectively between months 3 and 6.	Initial flatulence	TNFα decreased by 0.6pg/ml between baseline and month 6. IL-6 decreased by 3.7pg/ml and 3.9pg between baseline and months 3 and 6 respectively. hs-CRP decreased by 2.4mg/ml and 2.9mg/ml between baseline and months 3 and 6 respectively.	

19	Insulin, HOMA-IR and HOMA-B decreased in by 3.2µIU/dl, 1.5 and 7.2 in synbiotic group between baseline and week 8				
20	HbA1c decreased by 0.13% and FPG decreased by 10.23mg/dl between baseline and week 8				
21	HbA1c, HOMA-IR, FPG and Fasting insulin decreased by 0.5%, 1.7, 7.3mg/dL and 4.5 µg/mL respectively between baseline and week 12. QUICKI increased by 0.32 in the same period	Increase in BMI of 0.3kg/m2 and increase in weight of 0.7kg.		Hs-CRP and MDA decreased by 2,611ng/ml and 0.3µmol/L respectively between baseline and 12 weeks. TAC and GSH increased by 96mmol/L and 48µmol/L respectively between baseline and week 12.	
22					Increase in Bifidobacterium and 4 other taxa between baseline and week 12.
23		Triglycerides decreased by 8.95mg/dl and 16.69mg/dl in IG1 between baseline and months 3 and 6 respectively. Triglycerides decreased by 11.31mg/dl and 12.23mg/dl in IG2 between baseline and months 3 and 6 respectively.	Mild gastro complications: flatulence, dysphagia and dyspepsia		Increase in abundance of *Bacteriodes fragilis* to *E. coli* ratio in IG1 between baseline and month 6. Decrease in relative proportion of Firmicutes to Bacteroidetes in IG1 between baseline and month 6.
24					Changes to 1 phyla, 3 classes, 1 families, 7 genera and 17 species between baseline and week 8. Reversals to species associated with prediabetes

Table 2: Summary of significant clinical outcomes in the intervention groups (where P value <0.05)

Heterogeneity of reporting on significance and standard deviations

The majority of studies measured significance within group, comparing changes at different times in study to baseline figures, and significance compared to placebo and/or other interventions. However, some studies reported only on significance compared to placebo and/or other interventions. Whereas most studies reported on standard deviations for baseline and changes, some studies omitted these.

Three studies (6, 8 and 18) found improvements to insulin sensitivity. Homeostasis model assessment of insulin resistance (HOMA-IR) and fasting plasma glucose (FPG) improved in studies 6 and 18 with and fasting insulin also improving in study 6. Insulin sensitivity index (ISI) improved in study 8. This last study was unusual as its mean study age was considerably older at 66 years and all participants had abdominal obesity. Kijmanawat, Panburana, Reutrakul et al (2019) in a study of insulin resistance in gestational DM said that insulin resistance was improved by probiotics through several pathways including improvements to oxidative stress, gut permeability and increased secretion of incretins.

Other studies show that probiotics can be successful even if no lifestyle changes have been applied. For example, study 18 comprised participants who were not receiving lifestyle interventions to assist control of the disease with diet or exercise in a wealthy population with access to excess food.

However, that does not mean that probiotics necessarily are effective regardless of other factors. For example, the systematic review on glycaemic control by Ruan et al (2015) found evidence for glycaemic control with probiotics on participants receiving antidiabetic medication and surmised that glucose lowering effect occurred due to probiotic causing increased efficacy of antidiabetic medication. There were no studies in this review that could disprove this theory. There were no studies from prediabetic participants that found improvements in glycaemic control. Studies on the effect of probiotics on participants not taking antidiabetic medication would be very useful.

Within the studies that reported improvements, studies 5, 8 and 11 all featured two probiotic intervention groups in each study with significant results in only one of the intervention arms. Study 5 only found significant results in the 'live-strain' arm and not the heat-killed version, study 8 found significant results in the high-dose but not the low dose intervention and study 11 found significant results in the 5-strain probiotic but not the 3-strain probiotic. This shows that even within the same clinical trial two probiotic interventions could show different results so that more research needs to be done to calculate the correct formulas and dosages needed in such interventions.

Case control study (3) described increased efficacy of Metformin on glycaemic control and control of loose stools after co-biotic intervention of prebiotic inulin with antioxidant blueberry in one newly-diagnosed obese T2DM patient. A systematic review by Rao, Goa, Xu et al (2019) found inulin could improve glycaemic control in obese T2DM participants.

Synbiotics are also a promising intervention. Four studies on synbiotics measured outcomes on glycaemic control with significant improvements (15,19, 20 and 21). Three of the studies that led to improved outcomes involved the prebiotic inulin (15,19 and 21) while the fourth involved an unstated measurement of FOS (20), suggesting that inulin could play an important role.

On the other hand, a prebiotic called GOS produced adverse effects in one study. Study 10 where participants consumed 5.5g GOS over 12 weeks reported a significant deterioration in glucose effectiveness at zero insulin (GEZI). There were also significant increase in mean body fat. This finding has been supported by Lui, Li, Chen et al (2017) who found that supplementation with GOS led to adverse glycaemic outcomes in healthy young participants which was ascribed to GOS leading to a reduction in the butyrate-producing bacteria Ruminococcus despite increasing the abundance of Bifidobacterium.

Only 1 trial featured a very small number of T1DM (21) participants with the rest being T2 or prediabetic. Therefore there is no evidence that the interventions are effective or safe for T1DM patients. Overall, the effect of such interventions look promising for T2DM but more research is needed on the dosage, strains, and the timings of administrations with larger populations in the trials.

Biomarkers of Oxidative stress/ inflammation and gut permeability

Eight studies (4, 5, 7, 13, 15, 16, 18, 21) reported significant improvements to markers for oxidative stress, inflammation and/or gut permeability. Three studies were probiotic, 1 prebiotic and 4 synbiotic.

A systematic review of probiotics and synbiotics on inflammation, oxidative stress markers and markers for epithelial barrier integrity (Zheng, Guo, Jia et al 2019) found evidence for improvements in adult participants with T1 or T2DM. However, they found the results from all studies from Iran produced significant improvements which were not replicated in studies from other countries and cautioned against the validity of findings. In this review, four of the studies resulting in significant results were from Iran with the remaining four from Austria, Taiwan, Ukraine and Saudi Arabia, which does not accord with the conclusion reached by Zheng et. al.

Study 4, a synbiotic intervention, found a significant decrease in serum zonulin at 3 months but not at 6 months. Zonulin is a biomarker for gut barrier integrity and increases in zonulin have been correlated with loss of barrier function (Sturgeon and Fasano 2016) and has been found to be higher in newly-diagnosed T2DM and correlated with insulin resistance (Zhang, Zhang, Zheng et al 2014). A systematic review (Ramezani Ahmadi, Sadeghian, Alipour et al (2020) found synbiotics and probiotics reduced zonulin levels by protecting the epithelium and protecting against pathogenic bacteria. Three studies on probiotics found significant improvements in inflammatory markers (5,7,18) with decreases in proinflammatory cytokines Il-1β (5,7), TNF-α (7,18), Il-6(7,18) and hs-CRP (18). Increased levels of inflammatory markers have been found to be associated with microvascular and macrovascular complications of DM (Mankowska, Pollak and Sypniewska 2006) and probiotics have been found to have anti-inflammatory effects in chronic diseases though the pathways are not understood (Plaza-Diaz, Ruiz-Ojeda, Vilchez-Padial et al. 2017).

The remaining 4 studies from Iran looked at prebiotics (13), and synbiotics (15, 16, and 21). Study 13 found evidence for reductions in inflammation with decreases in hs-CRP and serum MDA as did synbiotic studies with improvements in Hs-CRP (15, 16, 21), and improvements in MDA (16, 21). A systematic review (McLoughlin, Berthon, Jensen et al 2017) found evidence for anti-inflammatory effects of prebiotics and synbiotics thought to be due to regulating the epithelial barrier

and increasing anti-microbial peptides. Three Iranian studies (15, 16, 21) also found evidence for improvements in oxidative stress with increases in reduced glutathione (GSH) (15,21), increase in NO (16) and increase in TAC (21). Oxidative stress is associated with the progression of DM (Giacco and Brownleee, 2010). A systematic review (Heshmati, Farsi, Shokri et al 2018) on effects of probiotics and synbiotics on oxidative stress found evidence for their use on increasing oxidative stability and improving antioxidant capabilities but found that the effects of probiotics within the synbiotic mix had the greatest effect.

Overall, there is evidence that probiotics and synbiotics do reduce oxidative stress, inflammation and gut permeability. While it is of concern that doubt has been raised about studies from Iran by Zhang et al, there are four non-Iranian studies from this review and other systematic studies that support the hypothesis that these interventions are effective.

Effect on lipids/anthropometric parameters/blood pressure

Eleven studies reported significant effects on lipids, blood pressure and anthropometric parameters, but with varying outcomes for subjects.

Five studies found significant improvements for lipids with probiotic supplementation (5, 12, 14, 18, 23), finding improvements in cholesterol (5, 14, 18 high density lipoproteins (12, 18), low density lipoproteins (14), and triglycerides (23). A systematic review (Gadelha and Bezerra 2019) on adult participants found probiotics significantly reduced total cholesterol, LDL, and triglycerides and increased HDL, with effects evident after 6 weeks of supplementation. The trials in this review finding evidence ranged in length of study from 6 weeks (12, 14) to 6 months (5, 18, 23). Lipid improving actions of probiotics are thought to result from bile acid synthesis (Sivamaruthi, Fern, Ismail et al 2020).

Four probiotic studies (1, 6, 7) found evidence for improvements to anthropometric parameters, female waist circumference (1), weight (6,7), and BMI (6,7). However, study 8 found weight and BMI increased in a lose dose probiotic intervention. A review (Mazloom, Siddiqi and Covasa, 2019) of animal and human trials of probiotics on obesity found improvements to weight and fat parameters in animal studies but like this review, inconsistent results for human ones which they attributed to differences in probiotic strains and the failure to identify the known pathways and synergistic relationships of specific strains.

Two studies (1, 5) on probiotics reported improvements to blood pressure parameters: SBP improved in both studies and mean BP also improved in study 5. A systematic review (Qi, Nie, and Zhang 2020) on effect of probiotics on BP found significant improvements in SBP but only for participants with DM or hypertension and that the improvements were only short lived for a maximum of 10 weeks. In study 5, both intervention groups had significant improvements in SBP at month 6.

A review of prebiotics (Nie, Chen, Hu et al. 2020) found that while animal trials reported improvements in body composition using GOS, these were not replicated in human trials and anti-obesity effects of GOS, might depend on whether its structure was α-GOS or β-GOS. However, the prebiotic case control study on effects of inulin and antioxidant resulted in weight loss for newly-diagnosed T2 patient. These conflicting results point to a need for more research.

The synbiotic studies in this review also had conflicting results. Two study arms found improvements in parameters following synbiotic intervention (4, 23) while one (21) reported deteriorations. Study 4 found significant decrease in hip circumference, however, large hip circumference is thought to be less of risk factor for metabolic diseases (Katz, Stevens, Truesdale et al. 2011) than waist or BMI measurement. Study 23 found significant reduction in triglycerides. This result was also found in a meta-analysis (Beserra, Fernandes, do Rosario et al 2014) on overweight and obese participants following supplementation with synbiotics. Study 21 featuring a cohort with chronic kidney disease (CKD) with higher mean age of cohort than other studies and found increases in weight and BMI following synbiotics supplementation. While many studies using synbiotics have found improvements, further research is needed to understand their effects on different groups.

There was no evidence for improvements using prebiotics in this review. Probiotic interventions showed some promising outcomes but with mixed results. Probiotics improved lipid profiles but had mixed results with anthropometric parameters. There was also a small amount of evidence for improvement in blood pressure parameters. There was little evidence for the use of synbiotics in this area.

Tolerability of interventions and adverse events

The Medicines for Human Use (Clinical Trials) Regulations 2004 defines adverse event as '…any untoward medical occurrence in a subject to who a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product'.

The consistency about reporting on safety and tolerability is not standardised. In this review, seventeen studies reported either no adverse events due to intervention, or that the product was well tolerated, or that adverse events were similar to placebo. These were probiotic arms of studies (5,6,9,12,14,17), prebiotic arms (2,10,22,24) and synbiotic arms (15,16,17,19,20,21,23). Some studies reported how many participants had withdrawn from the study due to adverse events (4,10, 13,18, 19,23), but this was restricted to a minority of studies. Other studies simply reported that the intervention was well tolerated or that no adverse events were attributed to intervention (2,5,6,9,11,12,14,15,16,17,19,20,21,22).

Common complaints relating to tolerability were minor GI disturbances including flatulence, mild abdominal pain, nausea and diarrhoea which occurred with equal frequency in intervention groups and placebo groups (1, 4, 7, 8, 11, 12). Studies specifically reporting adverse events often categorise them differently. Adverse events were noted as flatulence and diarrhoea (4), GI disturbance (10), severe GI symptoms (13), initial flatulence (18) and GI symptoms (23). In other studies these were not categorised as adverse events.

While only study 13 described the adverse effects as severe, the research community would benefit from a standardised taxonomy of adverse effects in this field to make the results comparable.

In addition, follow ups rarely occurred, which could have unearthed longer-term side effects of such interventions. Follow ups were reported in studies 4 (6 months post trial) and 5 (3 months post trial). Few studies recorded that they prompted participants for AE. None of the studies had an intervention beyond 6 months and sample sizes were small giving a low assurance of risk.

In terms of tolerability, side effects appeared to be temporary but more consistency in reporting and follow up assessments are needed if these interventions are to be considered safe.

Conclusion

Probiotics have been shown to have a positive effect on T2DM postprandial glycaemic control and insulin sensitivity, and have been shown to work in the short term at trials of only 6 weeks as well as longer trials of 6 months. There is no evidence for their use for T1DM patients. However, it is unclear if improvements are in increasing the efficacy of antidiabetic medication or in their own action. More trials are needed that should include DM participants on diet and exercise. Inulin, a prebiotic, has also resulted in clinical improvements when used alone or as a synbiotic.

In terms of biomarkers for oxidative stress, inflammation and gut permeability, there was evidence that probiotics and synbiotics can result in clinical improvements. Only 1 study on prebiotics found clinical improvements in this area.

In terms of anthropometric parameters, blood pressure or lipid profiles, there was no evidence for improvements using prebiotics in this review. Probiotics improved lipid profiles but had little success with anthropometric parameters. There were also a small number of studies with evidence for improvement in blood pressure parameters. There was no evidence for prebiotic supplementation and there was little evidence for the use of synbiotics in this area.

Overall, more studies are needed in this area from a wider range of countries and with larger cohorts. Future trials need to assess the influence of pro/pre/synbiotics in combination with and without drug therapy for diabetes. More research needs to be undertaken on prebiotics as only inulin at present appears to result in improvements.

There do not appear to be serious adverse effects with the interventions beyond short-term gastrointestinal upset though flatulence which might be unacceptable social side effect. Additional studies with follow ups are needed and more consistency is needed with reporting adverse effects.

References

1. Asemi Z, Khorrami-Rad A, Alizadeh SA, Shakeri H, Esmaillzadeh A (2014) Effects of synbiotic food consumption on metabolic status of diabetic patients: a double-blind randomized cross-over controlled clinical trial. *Clinical nutrition (Edinburgh, Scotland)* 33: 198-203.
2. Beserra BT S, Fernandes R, do Rosario VA, Mocellin M, Kuntz MG F, et al (2014) A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity . Clinical Nutrition 34: 845-858.
3. Bessac A, Cani PD, Meunier E, Dietrich G, Knauf C (2018) Inflammation and Gut-Brain Axis During Type 2 Diabetes: Focus on the Crosstalk Between Intestinal Immune Cells and Enteric Nervous System. *Frontiers in neuroscience* 12: 725.
4. Boussageon R, Pouchain D, Renard V (2017) Prevention of complications in type 2 diabetes: is drug glucose control evidence based? *British Journal of General Practitioners* 67: 85-87.
5. Canfora EE, van der Beek CM, Hermes GD A, Goossens GH, Jocken JW E, et al. (2017) Supplementation of Diet With Galacto-oligosaccharides Increases Bifidobacteria, but Not Insulin Sensitivity, in Obese Prediabetic Individuals. *Gastroenterology* 153: 87-97.
6. Carabotti M, Scirocco A, Maselli MA, Severi C (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of gastroenterology* 28: 203-209.
7. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ (2015) Dysbiosis of the gut microbiota in disease. *Microbial ecology in health and disease* 26: 26191.
8. de Goffau MC, Fuentes S, van den Bogert B, Honkanen H, de Vos WM, et al. (2014) Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. *Diabetologia* 57: 1569-1577.
9. Delzenne N, Cani PD (2011) Gut Microbiota and the Pathogenesis of Insulin Resistance. *Current Diabetes Report* 11: 154-159.
10. Den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, et al. (2013) The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 54: 2325-2340.
11. Ding S, Lund PK (2011) Role of intestinal inflammation as an early event in obesity and insulin resistance. *Current opinion in clinical nutrition and metabolic care* 14: 328-333.
12. Ebrahimi ZS, Nasli-Esfahani E, Nadjarzade A, Mozaffari-Khosravi H (2017) Effect of symbiotic supplementation on glycemic control, lipid profiles and microalbuminuria in patients with non-obese type 2 diabetes: a randomized, double-blind, clinical trial. *Journal of diabetes and metabolic disorders 16: .*23.
13. Faerch K, Hulmán A, Solomon TP J (2016) Heterogeneity of Pre-diabetes and Type 2 Diabetes: Implications for Prediction, Prevention and Treatment Responsiveness, *Current Diabetes Reviews* 12: 30-41.
14. Farrokhian A, Raygan F, Soltani A, Tajabadi-Ebrahimi M, Sharifi Esfahani M, et al. (2019)The Effects of Synbiotic Supplementation on Carotid Intima-Media Thickness, Biomarkers of Inflammation, and Oxidative Stress in People with Overweight, Diabetes, and Coronary Heart Disease: a Randomized, Double-Blind, Placebo-Controlled Trial. *Probiotics and antimicrobial proteins 11*: 133-142.

15. Firouzi S, Majid HA, Ismail A, Kamaruddin NA, Barakatun Nisak MY, et al. (2017) Effect of multi-strain probiotics (multi-strain microbial cell preparation) on glycemic control and other diabetes-related outcomes in people with type 2 diabetes: a randomized controlled trial. *European Journal of Nutrition* 56: 1535-1550.

16. Flint H, Scott K, Duncan S, Louis P, Forano E (2012) Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* 3: 289-306.

17. Fluitman KS, De Clercq NC, Keijser BJ, Visser M, Nieuwdorp M, et al. (2017) The intestinal microbiota, energy balance, and malnutrition: emphasis on the role of short-chain fatty acids. *Expert Review of Endocrinology & Metabolism* 12: 215-226.

18. Gadelha C, Bezerra AN (2019) Effects of probiotics on the lipid profile: systematic review. *Jornal Vascular Brasileiro 18*: e20180124.

19. Giacco F, Brownlee M. (2010) Oxidative Stress and Diabetic Complications. *Circulation Research* 107: 1058-1070.

20. Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, et al. (2016) Genetic Determinants of the Gut Microbiome in UK Twins. *Cell Host Microbe* 19: 731-743.

21. Gonai M, Shigehisa A, Kigawa I, Kurasaki K, Chonan O, et al. (2017) Galacto-oligosaccharides ameliorate dysbiotic Bifidobacteriaceae decline in Japanese patients with type 2 diabetes. *Beneficial Microbes* 8: 705-716.

22. Greenway F, Wang S, Heiman M (2014) A novel cobiotic containing a prebiotic and an antioxidant augments the glucose control and gastrointestinal tolerability of metformin: a case report. *Beneficial Microbes* 5: 29-32.

23. Grom LC, Coutinho NM, Guimaraes JT, Balthazar CF, Silva R, et al. (2020) Probiotic dairy foods and postprandial glycemia: A mini-review. Trends in Food Science & Technology 101: 165-171.

24. Gurung M, Li Z, You H, Rodrigues R, Jump D, et al. (2020) Role of gut microbiota in type 2 diabetes pathophysiology. *EBioMedicine* 51: 102590.

25. Heshmati J, Farsi F, Shokri F, Rezaeinejad M, Almasi-Hashiani A, et al. (2018) A systematic review and meta-analysis of the probiotics and synbiotics effects on oxidative stress. Journal of Functional Foods 46: 66-84.

26. Ho J, Nicolucci AC, Virtanen H, Schick A, Meddings J, et al. (2019) Effect of Prebiotic on Microbiota, Intestinal Permeability, and Glycemic Control in Children With Type 1 Diabetes, *The Journal of Clinical Endocrinology & Metabolism* 104: 4427-4440.

27. Horvath A, Leber B, Feldbacher N, Tripolt N, Rainer F, et al. (2019). Effects of a multispecies synbiotic on glucose metabolism, lipid marker, gut microbiome composition, gut permeability, and quality of life in diabesity: a randomized, double-blind, placebo-controlled pilot study. *European Journal of Nutrition* 59: 2969-2983.

28. Hsieh MC, Tsai WH, Jheng YP, Su SL, Wang SY, et al. (2018) The beneficial effects of Lactobacillus reuteri ADR-1 or ADR-3 consumption on type 2 diabetes mellitus: a randomized, double-blinded, placebo-controlled trial. *Scientific Reports* 8: 16791.

29. Kassaian N, *Feizi A, Aminorroaya A, Tajabadi Ebrahimi M., Norouzi A., & Amini, M.* (2019) Effects of Probiotics and Synbiotic on Lipid Profiles in Adults at Risk of Type 2 Diabetes: A Double-Blind Randomized Controlled Clinical Trial. Functional Foods in Health and Disease 9: 494-507.

30. Kassaian N, Feizi A, Aminorroaya A, Amini M (2019) Probiotic and synbiotic supplementation could improve metabolic syndrome in prediabetic adults: A randomized controlled trial. *Diabetes & metabolic syndrome 13*: 2991-2996.

31. Kassaian N, Feizi A, Rostami S, Aminorroaya A, Yaran,M, et al. (2020) the effects of 6 mo of supplementation with probiotics and synbiotics on gut microbiota in the adults with prediabetes: A double blind randomized clinical trial. Nutrition 79-80.

32. Katz EG, Stevens J, Truesdale KP, Cai J, Adair LS, et al. (2011). Hip circumference and incident metabolic risk factors in Chinese men and women: the People's Republic of China study. *Metabolic syndrome and related disorders 9*: 55-62.

33. Khalili, L, Alipour B, Asghari Jafar Abadi M, Faraji I, Hassanalilou T, et al. (2019) The Effects of Lactobacillus casei on Glycemic Response, Serum Sirtuin1 and Fetuin-A Levels in Patients with Type 2 Diabetes Mellitus: A Randomized Controlled Trial. *Iranian biomedical journal 23*: 68-77.

34. Kijmanawat A, Panburana P, Reutrakul S, Tangshewinsirikul C (2019) Effects of probiotic supplements on insulin resistance in gestational diabetes mellitus: A double-blind randomized controlled trial. *Journal of diabetes investigation 10*: 163-170.

35. Knight J, Bayram Weston Z, Nigam Y (2019) Gastrointestinal tract 6: the effects of gut microbiota on human health. *Nursing Times* 155: 46-50.

36. Kobyliak N, Falalyeyeva T, Mykhalchyshyn G, Kyriienko D, Komissarenko I (2018) Effect of alive probiotic on insulin resistance in type 2 diabetes patients: Randomized clinical trial. *Diabetes & metabolic syndrome 12*: 617-624.

37. Langdon A, Crook N, Dantas G (2016) The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome Medicine* 8: 39.

38. Lee B, Moon KM, Kim CY (2018) Tight Junction in the Intestinal Epithelium: Its Association with Diseases and Regulation by Phytochemicals. *Journal of Immunology Research vol 2018*: 2645465.

39. Litvak Y, Byndloss MX, Bäumler AJ (2018) Colonocyte metabolism shapes the gut microbiota. *Science* (New York, N.Y.) 362: 1017.

40. Liu F, Li P, Chen M, Luo Y, Prabhakar M, et al. (2017) Fructooligosaccharide (FOS) and Galactooligosaccharide (GOS) Increase Bifidobacterium but Reduce Butyrate Producing Bacteria with Adverse Glycemic Metabolism in healthy young population. *Scientific reports 7*: 11789.

41. Liu H, Wang J, He T, Becker S, Zhang G, et al. (2018) Butyrate: A Double-Edged Sword for Health? *Advances in Nutrition* 9: 21-29.

42. Lloyd Price J, Abu Ali G, Huttenhower C (2016) The healthy human microbiome. *Genome Medicine* 8: 51.

43. Luca M, Di Mauro M, Di Mauro M, Luca A (2019) Gut Microbiota in Alzheimer's Disease, Depression, and Type 2 Diabetes Mellitus: The Role of Oxidative Stress. *Oxidative Medicine Cellular Longevity* 1-10.

44. McLoughlin RF, Berthon BS, Jensen ME, Baines KJ, Wood LG (2017) Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. *The American journal of clinical nutrition 106*: 930-945.

45. Mankowska A, Pollak J, Sypniewska G (2006) Association of C-Reactive Protein and Other Markers of Inflammation with Risk of Complications in Diabetic Subjects. *EJIFCC*, *17*: 8-11.

46. Mazloom K, Siddiqi I, Covasa M (2019) Probiotics: How Effective Are They in the Fight against Obesity?. *Nutrients 11*: 258.

47. Mirmiranpour H, Huseini HF, Derakhshanian H, Khodaii Z, Tavakoli Far B (2020) Effects of probiotic, cinnamon, and synbiotic supplementation on glycemic control and antioxidant status in people with type 2 diabetes; a randomized, double-blind, placebo-controlled study. *Journal of Diabetes & Metabolic Disorders* 19: 53-60.

48. Mobini R, Tremaroli V, Ståhlman M, Karlsson F, Levin M, et al. (2017) Metabolic effects of Lactobacillus reuteri DSM 17938 in people with type 2 diabetes: A randomized controlled trial. *Diabetes, Obesity & Metabolism* 19: 579-589.

49. Nikbakht E, Khalesi S, Singh I, Williams L, West T, et al. (2018) Effect of probiotics and synbiotics on blood glucose: A systematic review and meta-analysis of controlled trials. *European Journal of Nutrition* 57: 95-106.

50. Nishimura R, LaPorte RE, Dorman JS, Tajima N, Becker D, et al. (2001) Mortality Trends in Type 1 Diabetes. *Diabetes Care* 24: 823-827.

51. Palacios T, Vitetta L, Coulson S, Madigan CD, Lam YY, et al. (2020) Targeting the Intestinal Microbiota to Prevent Type 2 Diabetes and Enhance the Effect of Metformin on Glycaemia: A Randomised Controlled Pilot Study. *Nutrients 12*: 1-15.

52. Paun A, Yau C, Danska JS (2017) The Influence of the Microbiome on Type 1 Diabetes. *J Immunology* 198: 590-595.

53. Pedersen C, Gallagher E, Horton F, Ellis R, Ijaz U, et al. (2016) Host-microbiome interactions in human type 2 diabetes following prebiotic fibre (galacto-oligosaccharide) intake. *British Journal of Nutrition* 116: 1869-1877.

54. Perraudeau F, McMurdie P, Bullard J, Cheng A, Cutliffe C, et al. (2020) Improvements to postprandial glucose control in subjects with type 2 diabetes: A multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulation. *BMJ Open Diabetes Research and Care* 8: 1-9.

55. Plaza Díaz J, Ruiz Ojeda FJ, Vilchez Padial LM, Gil A (2017) Evidence of the Anti-Inflammatory Effects of Probiotics and Synbiotics in Intestinal Chronic Diseases. *Nutrients 9*: 555.

56. Pussinen PJ, Havulinna AS, Lehto M, Sundvall J, Salomaa V (2011) Endotoxemia is associated with an increased risk of incident diabetes. *Diabetes Care* 34: 392-397.

57. Qi D, Nie X, Zhang J (2020) The effect of probiotics supplementation on blood pressure: a systemic review and meta-analysis. *Lipids Health Dis* **19:** 79.

58. Rajkumar H, Kumar M, Das N, Kumar SN, Challa HR et al. (2015) Effect of probiotic lactobacillus salivarius UBL S22 and prebiotic fructo-oligosaccharide on serum lipids, inflammatory markers, insulin sensitivity, and gut bacteria in healthy young volunteers: a randomized controlled single-blind pilot study. *Journal of cardiovascular Pharmacology and Therapeutics*, Volume 20: 289-298.

59. Rao M, Gao C, Xu L, Jiang L, Zhu J, et al. (2019) Effect of Inulin-Type Carbohydrates on Insulin Resistance in Patients with Type 2 Diabetes and Obesity: A Systematic Review and Meta-Analysis. *Journal of diabetes research* 5101423.

60. Ramezani Ahmadi A, Sadeghian M, Alipour M, Ahmadi Taheri S, Rahmani S, et al. (2020) The Effects of Probiotic/Synbiotic on Serum Level of Zonulin as a Biomarker of Intestinal Permeability: A Systematic Review and Meta-Analysis. *Iranian Journal of Public Health 49*: 1222-1231.

61. Razmpoosh E, Javadi A, Ejtahed HS, Mirmiran P, Javadi M, et al. (2019) The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A randomized placebo controlled trial. *Diabetes & Metabolic Syndrome* 13: 175-182.

62. Rodriguez Gutierrez R, Montori VM (2016) Glycaemic Control for Patients with Type 2 Diabetes Mellitus: Our Evolving Faith in the Face of Evidence. *Circulation* 134: 1685.

63. Roshanravan N, Roshanravan N, Mahdavi R, Alizadeh E, Ghavami A, et al. (2017) The effects of sodium butyrate and inulin supplementation on angiotensin signaling pathway via promotion of *Akkermansia muciniphila* abundance in type 2 diabetes; A randomized, double-blind, placebo-controlled trial. *Journal of cardiovascular and thoracic research 9*: **183-190.**

64. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, et al. (2018) Environment dominates over host genetics in shaping human gut microbiota. *Nature* 555: 210-215.

65. Ruan Y, Sun J, He J, Chen F, Chen R., et al. (2015) Effect of Probiotics on Glycemic Control: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials. *PloS ONE* 10: 0132121.

66. Sabico S, Al Mashharawi A, Al Daghri NM., Wani K, Amer OE, et al. (2019) Effects of a 6-month multi-strain probiotics supplementation in endotoxemic, inflammatory and cardiometabolic status of T2DM patients: A randomized, double-blind, placebo-controlled trial. *Clinical nutrition (Edinburgh, Scotland) 38*: 1561-1569.

67. Salim SY, Kaplan GG, Madsen KL (2014) Air pollution effects on the gut microbiota: a link between exposure and inflammatory disease. *Gut Microbes* 5: 215-219.

68. Sekirov I, Russell S, Antunes L, Finlay B (2010) Gut Microbiota in Health and Disease. *Physiological Reviews* 90: 859-904.

69. Sivamaruthi BS, Fern LA, Ismail DS, Chaiyasut C (2020) The influence of probiotics on bile acids in diseases and aging, Biomedicine & Pharmacotherapy, Volume 128.

70. Soleimani A, Motamedzadeh A, Zarrati Mojarrad M, Bahmani F, Amirani E, et al. (2019) The Effects of Synbiotic Supplementation on Metabolic Status in Diabetic Patients Undergoing Hemodialysis: a Randomized, Double-Blinded, Placebo-Controlled Trial. *Probiotics and antimicrobial proteins 11*: 1248-1256.

71. Sterne JA C, Savović J, Page MJ, Elbers RG, Blencowe NS et al. (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* **366**: l4898.

72. Sturgeon C, Fasano A (2016) Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue barriers 4*: e1251384.

73. Suez J, Zmora N, Zilberman Schapira G, Mor U, Dori-Bachash M, et al. (2018) Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. *Cell (Cambridge)* 174: 1406-1423.

74. Tajadadi Ebrahimi M, Bahmani F, Shakeri H, Hadaegh H, Hijijafari M, et al. (2014) Effects of daily consumption of synbiotic bread on insulin metabolism and serum high-sensitivity C-reactive protein among diabetic patients: a double-blind, randomized, controlled clinical trial. *Annals of nutrition & metabolism 65*: 34-41.

75. Tenorio Jiménez C, Martínez-Ramírez MJ, Gil A, Gómez Llorente C (2020) "Effects of Probiotics on Metabolic Syndrome: A Systematic Review of Randomized Clinical Trials." *Nutrients* 1: 124.

76. Thaiss CA, Levy M, Grosheva I, Zheng D, Soffer E, et al. (2018) Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. *Science* (New York, N.Y.) 359: 1376-1383.

77. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, et al. (2018) Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *Lancet Diabetes Endocrinol* 6: 122-129.

78. Tonucci LB, Olbrich Dos Santos KM, Licursi de Oliveira L, Rocha Ribeiro SM, Duarte Martino HS (2017) Clinical application of probiotics in type 2 diabetes mellitus: A randomized, double-blind, placebo-controlled study. *Clinical Nutrition* 36: 85-92.

79. Valdes AM, Walter J, Segal E, Spector T (2018) Role of the gut microbiota in nutrition and health. *BMJ* 361: 2179.

80. Vaarala O, Atkinson MA, Neu J (2008) The "perfect storm" for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. *Diabetes* 57: 2555-2562.

81. Wolosin JD, Edelman SV (2000) Diabetes and the Gastrointestinal Tract. *Clinical Diabetes* 18(4).

82. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ (2006) Colonic health: fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology* Mar 40: 235-243.

83. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, et al. (2017) Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nature Medicine* 23: 850-858.

84. Xu Y, Zhou H, Zhu Q (2017) The Impact of Microbiota-Gut-Brain Axis on Diabetic Cognition Impairment. *Frontiers in Aging Neuroscience* 9: 106.

85. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, et al. (2015) Personalized Nutrition by Prediction of Glycemic Responses. *Cell* 163: 1079-1094.

86. Zhang D, Zhang L, Zheng Y, Yue F, Russell RD, et al. (2014) Circulating zonulin levels in newly diagnosed Chinese type 2 diabetes patients. *Diabetes research and clinical practice 106*: 312-318.

87. Zheng HJ, Guo J, Jia Q, Huang YS, Huang WJ, et al. (2019) The effect of probiotic and synbiotic supplementation on biomarkers of inflammation and oxidative stress in diabetic patients: A systematic review and meta-analysis of randomized controlled trials. *Pharmacological research 142*: 303-313.