Introduction
The human intestinal microbiota is a complex interplay composed of more than 1000 different species of bacteria, fungi, archaea and viruses. It is also estimated that the microbial gene pool is 100-fold greater than that of the human genome, so the relevance to human health is unsurprising. There is increased interest in the gut microbiota in human disease, with relationships elucidated in all facets of human medicine often far distant from the gastrointestinal tract. Animal models were the first to highlight a change in the gut bacteria in experimental obesity and type 2 diabetes (T2D), which could be predictably triggered by high-fat feeding. However, a recent large meta-analysis specifically looking at the relationship between the gut microbiota and human T2D pathophysiology has highlighted the consistency, complexity and contradictions within the data and, importantly, the relative lack of interventional trials linked to clinical end-points. Understanding and harnessing the microbiota as a treatment modality for conditions such as T2D is still in its infancy.

Disruption to the intestinal barrier can lead to an inflammatory cascade
The gut microbiota have an important role in humans and other animals in terms of complex energy salvage mechanisms. The gut epithelium therefore must form an essential barrier function by preventing the microbiota and their potentially pro-inflammatory products from gaining access from the gut lumen to the internal milieu. This may occur through the paracellular pathway between the intestinal cells, with tight junctions (tricellular junction; TCJ) being the rate limiting structure regulating the permeability of the intestinal barrier. Paracellular transport through TCJs can be modified through cell alterations in the architecture and expression of TCJ proteins. Butyrate, a C-4 short-chain fatty acid (SCFA) produced via glycolysis during the microbial fermentation of undigested carbohydrate, has been shown to be a key facilitator in the assembly of the TCJ via activation of AMP-activated protein kinase. Therefore, bacterial species known to be butyrate-producers or dietary components that will enhance the molar ratio of butyrate production, would be considered as important in maintaining an effective gut barrier.

Increased permeability of the gut barrier can result in an increased passage of bacterial products into the systemic circulation, including lipopolysaccharide (LPS), a major outer wall constituent of Gram-negative bacteria such as...
**Enterobacteriaceae** and **Bacteroides** (Figure 1). While only small amounts of LPS usually pass into the portal circulation, sufficient to stimulate an immune response to infection, if gut permeability is increased over an extended period, this will over-expose key metabolic tissues such as the liver and adipose tissue to this pathogen-associated molecule.\(^3\) The main cellular receptor for LPS is the hetero-receptor, consisting of CD14, Toll-like receptor 4 (TLR-4), MD2 and LPS-binding protein (LBP). With all of these components in place in the liver, LPS can activate a potent pro-inflammatory signalling cascade of cytokines (IL-1\(\beta\), TNF, IL-6) and chemokines (IL-8). This low-grade inflammation is implicated in the development of insulin resistance.

**Evidence for gut barrier dysfunction in type 2 diabetes**

Although animal models have provided most of the evidence base linking impaired gut barrier function, activation of inflammation signalling pathways and progression of IR, there are now increasing data supporting this paradigm in human T2D. Both functional increases in intestinal permeability\(^4\) and increased blood levels of zonulin, a protein that modulates the disassembly of TJ proteins,\(^5,6\) have been reported. Blood levels of LPS have also been shown to be consistently higher in T2D even when compared to matched BMI controls\(^7\) and, more recently, a blood-derived permeability risk score combining measurements of LPS, intestinal fatty acid binding protein (iFABP) and LBP was also found to be independently predictive of T2D.\(^8\)

**Diet is the primary modifiable risk factor for both dysbiosis and a healthy gut barrier**

Although more fundamental factors, such as genetics, life stage, early-life nutrition and prior/current drug exposure impact on the adult gut microbiota, the adult dietary pattern is also very important (Figure 2). All components of the diet have an impact on the gut microbiota; however, the balance of macronutrients, in addition to the influence of the individual components are likely important and may be more relevant in terms of dietary guidelines.

Dietary protein is relevant, or more importantly the carbohydrate to protein ratio as a substrate for the microbiota. Carbohydrates are the preferred fuel source for the microbiota. Carbohydrates are the preferred fuel source for the microbiota, but in circumstances where carbohydrate substrates become depleted, bacterial proteases and ureases break down proteins to peptides and amino acids, which can be fermented to branched-chain fatty acids (BCFA) and compounds including amines, indoles, phenol and ammonia. These fermentation by-products decrease the transport, uptake and oxidation of butyrate by the colonocyte,\(^9\) in addition to leading to changes in the composition of the gut microbiota with a reduction in *Bifidobacterium* and butyrate-producing species such as *Blautia*, and *Roseburia*.\(^{10}\) Protein fermentation by-products therefore not only decrease cellular integrity and gut barrier function directly, but also indirectly by inhibiting butyrate production and bioavailability. Variation in protein intake has been consistently associated with effects on gut barrier function in translatable animal models of the human gut, and recent studies in T2D also show increased levels of zonulin in response to an increased intake of protein.\(^{11}\) The potential effects of high-protein diets on the host–microbiome relationship have
been recently reviewed by Blachier et al.; however, much more evidence is required, specifically for T2D and the potential role of different protein sources and the increasing use of carbohydrate-restricted diets.

Directly targeting the gut microbiota through diet: the concept of a prebiotic

The concept of a ‘prebiotic’ was first defined by Gibson and Roberfroid in 1995 as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Initially, these species were confined to *Lactobacillus* and/or *Bifidobacterium* spp but now include other taxa including *Roseburia* spp but now include other taxa including *Faecalibacterium* and *Eubacterium* spp but now include other taxa.

The concept of the prebiotic is also evolving as the evidence base develops, with the updated consensus from 2017 as a ‘substrate that is selectively utilised by host-microbial organisms conferring a health benefit’, an effect no longer confined to oral administration. Initially, all prebiotics were carbohydrates (typically also fulfilling the CODEX Alimentarius definition of dietary fibre) but other substrates such as polyphenols (found in foods such as tea, cocoa, wine and coffee) and polyunsaturated fatty acids (PUFA) may now fit into this expanded definition.

The carbohydrate prebiotics are primarily composed of inulin, fructo-oligosaccharide (FOS) and galacto-oligosaccharide (GOS). Linear chains connected with β-(2-1) glycosidic bonds and so resistant to human enzymatic digestion in the small intestine. As such, the consumption of these compounds has no acute effect on blood glucose levels or stimulation of insulin secretion. They are, however, readily degraded by β-fructanase and β-galactosidase enzymes, which are prevalent in *Bifidobacterium*. Inulin, FOS and GOS are shown consistently to enrich *Lactobacillus* and/or *Bifidobacteria* spp. Due to their similar structure, inulin and FOS are often referred to by the term ‘inulin-type fructans’ (ITFs) and these compounds are by far the most studied prebiotics in humans.

ITFs are found naturally in over 36,000 varieties of plant species; however, in some plants, inulin is used as a storage carbohydrate instead of starch making these rich dietary sources of these oligosaccharides (Table 1). ITFs are also extensively used as industrial food ingredients in their extracted and synthesised form, used to replace sugar, fats and flour in processed goods for energy reduction or to produce foods with a healthier profile for the consumer. However, most clinical studies in humans have been through the direct use of supplements rather than with prebiotic-rich foods.

<table>
<thead>
<tr>
<th>Dietary source</th>
<th>Inulin-type fructan (g/100g)</th>
<th>Serving</th>
<th>Per typical serving size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana (raw)</td>
<td>1.0</td>
<td>Medium fruit</td>
<td>1.3</td>
</tr>
<tr>
<td>Asparagus (boiled)</td>
<td>3.4</td>
<td>5 spears</td>
<td>3.1</td>
</tr>
<tr>
<td>Dried chicory root</td>
<td>64.5</td>
<td>2 tablespoons</td>
<td>7.1</td>
</tr>
<tr>
<td>Garlic (raw)</td>
<td>17.5</td>
<td>Single clove</td>
<td>0.5</td>
</tr>
<tr>
<td>Globe artichoke</td>
<td>4.8</td>
<td>Small</td>
<td>6.1</td>
</tr>
<tr>
<td>Jerusalem artichoke</td>
<td>31.5</td>
<td>1 cup</td>
<td>47.3</td>
</tr>
<tr>
<td>Leeks</td>
<td>11.7</td>
<td>Small</td>
<td>14.5</td>
</tr>
<tr>
<td>Onions (cooked)</td>
<td>6.0</td>
<td>1 tablespoon</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 1. Typical sources of inulin-type fructans in the diet, expressed per serving size. Data adapted from Moshfegh et al.

Evidence for the efficacy of prebiotics for improving glycaemic control in type 2 diabetes

There has been a considerable evidence base in animal models for the potential of prebiotics to impact on metabolic effects relevant to T2D, such as obesity, hepatic steatosis, blood lipid levels, inflammation and insulin resistance; however, confirmatory evidence in humans has been slow to catch up. To date most work in the area is still in healthy humans without a diagnosis of T2D; however, it is not straightforward to simply translate efficacy directly to those with T2D. The relationship between prebiotics and the microbiota is confounded not only by the dysbiosis associated with T2D but also the important confounding effects and antibiotic-like actions of ubiquitous medications such as metformin.

The recent publication of three separate meta-analyses, including those with T2D, are now beginning to shed light in this important and understudied area. The most robust of these analyses by Wang et al. which included 34 randomised controlled trials (RCTs) in 1346 participants spread across healthy, obese and T2D groups reported that prebiotic supplementation led to a 0.58% reduction in HbA1c (95% CI -0.83, -0.32%) with little heterogeneity between studies. There has been an increasing number of individual studies which permitted a sub-group analysis to tease out details with respect to dosage and length of supplementation. This established that a daily dose of ≥10g/day supplementation was required for the most significant effects on glucose control. The supplements were also more effective in improving glucose control when administered into liquids rather than solids, which raises important questions for the consumption patterns of these prebiotics in real-world situations and understanding the impact of the food matrix.

The meta-analyses by Mahboobi et al. and most recently by Zhang et al., although smaller, were able to investigate the effects of prebiotics in T2D as a discrete grouping. A similar pattern emerges; in the Mahboobi study, there was a 0.47% reduction in HbA1c (95% CI -0.77, 0.21) and a reduction in the fasting
blood glucose (FBG) of -0.7mmol/L (95% CI -0.9, -0.5). In the more recent Zhang study, there was a 0.69% reduction in HbA1c (95% CI -0.92, -0.46), a reduction in FBG of 0.55mmol/L (95% CI -0.73, -0.36) and also a reduction in HOMA-IR of 0.81 units (95% CI -1.59, -0.03). These reductions in FBG and HOMA-IR were not documented in the Wang study; however, this was not exclusive to T2D and included individuals with FBG within the pre-diabetic range. The different analyses, however, are consistent in determining an optimal timescale of more than six to eight weeks of prebiotic intake for improvements to glycaemia.

Although these three recent meta-analyses are important first steps in investigating the efficacy of prebiotics in T2D, they are limited in terms of study number, relative size of individual studies, limited geographical area of included trials and, most importantly perhaps, the lack of microbiota data linked to reported improvements in glycaemic control. Without an associated change in the microbiota, can these improvements in glycaemia be attributed directly to the microbiota? ITF supplementation has indeed been shown to have a moderate effect on the faecal microbiota in T2D with a documented bifidogenic effect23 but without changes to overall diversity. However, this is not a universal finding and relatively few studies have investigated the metabolic effects of prebiotics in T2D have attempted to report changes in gut bacteria. This issue was addressed in a systematic review by Houghton et al.21 who examined the effectiveness of lifestyle interventions (including prebiotics) that target the microbiota, in combination with changes in glucose control in adults with T2D. Statistically significant differences in gut microbiota variables are consistently found in T2D studies and correlations can be made with markers of glycaemic control in individual studies; however, changes in the relative abundances of bacteria at the genus level such as Bifidobacterium, Roseburia and Lactobacillus, the key components often attributed to the prebiotic effect, are not always reported.22

### Most prebiotics are also non-viscous dietary fibres: can we or should we separate the two?

If a prebiotic carbohydrate cannot be demonstrated to change the microbiota in a way consistent with the accepted definition, are they simply exerting their effects as a dietary fibre? Ultimately, does it matter if a clinical effect can be achieved, whether this is through a change in the microbiota or simply due to enhanced microbial activity due to increasing carbohydrate substrate availability? Dietary fibres are carbohydrates which are not digested in the small intestine of humans and enter the large intestine. Most prebiotic carbohydrates fall within this definition. There have been numerous clinical studies revealing the important metabolic effects of dietary fibre ingestion, with many aspects potentially important for T2D. Of particular note are: (i) energy dilution and increased bulk of the diet leading to a reduction in energy intake; (ii) the systemic metabolism of the SCFA acetate and propionate with insulin sensitising effects in peripheral tissues; and (iii) the potential of SCFA to stimulate the release of hormones such as GLP-1 with effects on appetite and glucose metabolism.

Perhaps the most substantial and robust evidence for the effects of dietary fibre (all types combined) in the management of diabetes (all types) comes from the recent meta-analysis by Reynolds et al.25 In this large analysis, with data taken from 45 clinical trials of differing design, significant effects on glucose control could be attributed to the inclusion of additional fibre to the diet. These improvements credited to fibre ingestion per se could be used as a benchmark with which to compare the efficacy of prebiotic interventions: HbA1c -2.00mmol/mol (95% CI -3.0, -1.0), fasting glucose -0.56mmol/L (95% CI -0.73, -0.38), HOMA-IR -1.24 (95% CI -1.72, -0.76). The type of fibre did not appear to influence the extent of the improvements. The study concluded that total dietary fibre should be at the level of 35g/day for glycaemic benefit in T2D, which is higher than the 30g/day recommended by the recent Scientific Advisory Committee on Nutrition Carbohydrates and Health report for the general population. However, as the actual fibre intake in the UK is estimated to be 17g/day (UK Biobank), filling this fibre gap, with either prebiotic or non-prebiotic sources, would be beneficial for glycaemic management.

### Variability in the response between individuals: precision nutrition?

Within nutrition, the concept of responders and non-responders (or indeed understanding the variability of a particular response) to dietary interventions is an active research area. Unlike the preclinical models that are often used to establish the mechanisms of action, human populations are typically heterogeneous at the microbial, genetic, molecular, physiological and behavioural levels. The clinical effects of prebiotics within T2D have the potential to vary widely between individuals and to date most of the studies have been too small to suitably account for the substantial variability in the baseline microbiota. Of course, this defect can be partially resolved by conducting meta-analyses; however, it will not be truly resolved until the microbiota is routinely measured and reported from all prebiotic interventions, and larger trials of extended duration are conducted. Despite this limitation, several variables have been proposed as being potential sources of variability that predict which individuals will respond to prebiotic interventions.

It is likely that a major predictor will be the baseline microbial composition. In simple terms, microbial utilisation of a prebiotic can only occur if the appropriate bacteria are already present within the host. Baseline microbial diversity and more specifically Bifidobacteria concentration may be predictive, with a lower initial baseline level of Bifidobacteria predicting a greater bifidogenic response to prebiotic intake.24 Recent work using humanised-mice is attempting to identify elements of the microbial signature in individuals that show clinical benefit from prebiotic interventions.25 What appears likely from this work, and previous work...
looking at dietary fibre interventions, is that no one bacterium will be predictive, but rather a consortium of several species will drive the clinical effects. Baseline diet and biological sex have also been highlighted as important in predicting a beneficial response to prebiotic intake. From the meta-analysis by Wang et al., female participants were found to be more responsive in terms of improvements in glycaemic control compared to their male counterparts, although relative changes to the microbiota are not reported. Healthy women are shown to have a greater microbial diversity and exhibit lower plasma LPS and serum zonulin when compared to men, which would be consistent with improved barrier function. However, understanding any sexual dimorphism in either the microbial and/or glycaemic response to prebiotics specific to T2D warrants further investigation.

Conclusion
There is increasing evidence that prebiotics can have a clinical benefit for glycaemic control in T2D; however, due to the nature and design of most studies it is not clear whether these effects are due to modification of the microbiota or through increasing substrate availability for fermentation. There is a considerable variability in the individual response to prebiotics, both microbial and physiological, and future work will focus on both understanding this variability to specifically target individuals who will benefit

References