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Effect of fructans, prebiotics and fibres on the human gut microbiome assessed by 16S rRNA-based approaches: a review

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REVIEW ARTICLE

Abstract

The inherent and diverse capacity of dietary fibres, nondigestible oligosaccharides (NDOs) and prebiotics to modify the gut microbiota and markedly influence health status of the host has attracted rising interest. Research and collective initiatives to determine the composition and diversity of the human gut microbiota have increased over the past decade due to great advances in high-throughput technologies, particularly the 16S ribosomal RNA (rRNA) sequencing. Here we reviewed the application of 16S rRNA-based molecular technologies, both community wide (sequencing and phylogenetic microarrays) and targeted methodologies (quantitative PCR, fluorescent *in situ* hybridisation) to study the effect of chicory inulin-type fructans, NDOs and specific added fibres, such as resistant starches, on the human intestinal microbiota. Overall, such technologies facilitated the monitoring of microbiota shifts due to prebiotic/fibre consumption, though there are limited community-wide sequencing studies so far. Molecular studies confirmed the selective bifidogenic effect of fructans and galactooligosaccharides (GOS) in human intervention studies. Fructans only occasionally decreased relative abundance of *Bacteroidetes* or stimulated other groups. The sequencing studies for various resistant starches, polydextrose and beta-glucan showed broader effects with more and different types of gut microbial species being enhanced, often including phylotypes of *Ruminococcaceae*. There was substantial variation in terms of magnitude of response and in individual responses to a specific fibre or NDO which may be due to numerous factors, such as initial presence and relative abundance of a microbial type, diet, genetics of the host, and intervention parameters, such as intervention duration and fibre dose. The field will clearly benefit from a more systematic approach that will support defining the impact of prebiotics and fibres on the gut microbiome, identify biomarkers that link gut microbes to health, and address the personalised response of an individual's microbiota to prebiotics and dietary fibres.

Keywords: nutrition, intestine, inulin, microbiota, health

1. Introduction

Dietary fibres are acknowledged worldwide for their positive impact on health and well-being. Besides the well-known benefits on improved digestive health or bowel function, there are novel insights for the role of fibres in preventing obesity, reducing stress, supporting immunity, amongst other benefits (Canfora *et al.*, 2015; Koh *et al.*, 2016; Slavin, 2013; Stephen *et al.*, 2017; Van de Wouw *et al.*, 2018). The definitions of dietary fibres differ between countries or regions and include chemical properties as well as physiological effects, and some regions have specific definitions for isolated or synthetic fibres as opposed to those inherent in foods. The US Food and Drug Administration (FDA) has recently revised the nutrition facts label for foods and dietary supplements, and included a definition for the isolated/synthetic fibres, i.e. '*... isolated or synthetic non-digestible carbohydrates (with three or more monomeric units) that FDA determines to have a physiological effect that is beneficial to human health*' (FDA, 2016). Thus, the non-digestible carbohydrates must go through an US FDA approval procedure to be labelled as dietary fibre in the US. The European Union also has a fibre definition: '*carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine*' and *'which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence'* (EC, 2011). A CODEX definition for fibre was established in 2010 (FAO/WHO, 2010). Some nondigestible oligosaccharides (NDOs), i.e. saccharide polymers containing a small number of monosaccharides (typically 3-10), may also be classified as fibres depending on the country's regulation. A specific subset of fibres or NDOs can selectively stimulate certain gut microbiota species and those fibres are termed as prebiotics, a term coined in 1995 (Gibson and Roberfroid, 1995). The most recent definition of prebiotic by International Scientific Association of Probiotics and Prebiotics (ISAPP) is *'a substrate that is selectively utilised by the host microorganisms conferring a health benefit'* (Gibson *et al.*, 2017). There are increasing numbers of (potential) dietary fibres and prebiotics on the food market, which may be isolated from plants or manufactured by enzymatic and/or chemical means. To date, inulin-derived fructans from the chicory root are among the most thoroughly studied fibres recognised by ISAPP as prebiotic (Gibson *et al.*, 2017).

Since the early days of prebiotic research, the use of prebiotic fibres has been commonly associated with promoting growth of *Actinobacteria*, mainly the genus *Bifidobacterium* in the human colon. In the initial studies on the microbial effects of inulin-type fructans, the focus has been on the bifidogenic effect, as this appeared to be the most prominent outcome based on cultivation (Gibson and Roberfroid, 1995; Hidaka *et al.*, 1986). Introduction of culture independent, 16S ribosomal RNA (rRNA)-based methods and high-throughput sequencing techniques allowed for more extensive examination of the microbial community and the impact of dietary fibres (Falony *et al.*, 2016; Hornung *et al.*, 2018; Shetty *et al.*, 2017). Application of omics approaches together with mechanistic studies pointed to existence of microbial networks and cross-feeding which explains the involvement of other colonic bacteria that convert lactate and acetate into butyrate, notably those belonging to the genera *Anaerostipes*, *Anaerobutyricum* (previously *Eubacterium hallii*), *Eubacterium rectale* and *Ruminococcus bromii* (Belenguer *et al.*, 2006; Duncan *et al.*, 2004; Shetty *et al.*, 2018; Venkataraman *et al.*, 2016). Thus, the wider community approach to study different aspects of fibre fermentation and prebiotic effects might be necessary to establish a link between microbiota composition and activity on the one hand, and host health on the other (Delcour *et al.*, 2016).

A vast number of microbial species inhabits the human colon, mainly within bacterial phyla *Bacteroidetes* and *Firmicutes*, while minor phyla include *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, besides the *Archaea* kingdom (King *et al.*, 2019; Shetty *et al.*, 2017). There is increasing evidence that individual variation in the colonic microbiota predisposes consumers to respond differently to the same diets, as well as to dietary fibres. Remarkably, obese or overweight individuals who had a reduced gut microbial gene richness, presented more pronounced impaired metabolism and low-grade inflammation (Le Chatelier *et al.*, 2013). A subsequent dietary intervention with proteins and fibres including inulin improved the low gene richness and clinical phenotypes in the individuals, although, it was less effective for reducing inflammation parameters in individuals with lower gene richness (Cotillard *et al.*, 2013). Analyses of several intervention studies on fibre/resistant starch/fructan revealed for the first time potential microbial biomarkers or signatures for dietary responsiveness in obese individuals with impaired metabolic health (Korpela *et al.*, 2014a; Salonen *et al.*, 2014). A similar approach using gut microbiota signatures from an 800 person cohort, was applied in the development of an algorithm that predicted personalised diets to successfully lower elevated post-meal glucose responses (Zeevi *et al.*, 2015).

Research on 16S rRNA-based methods for investigating the effects of a variety of specific fibres, NDOs and prebiotic ingredients with emphasis on chicory-derived fructans on the human gut microbiota was first discussed in a workshop with experts in Chicago in 2016. The focus was on studies with children to adult human subjects, and with either specific added fibres or prebiotics. The outcome of the workshop forms the basis for this review. Although, it was recognised that there was considerable information to be learned from *in vitro* and animal research, the most relevant data can be derived from human studies. The main objective here is to review existing knowledge on the impact of the different added dietary fibres, NDOs and prebiotics on the diversity of the human faecal microbiota using various culture independent, 16S rRNA based technologies.

The focus of this review is a description of human clinical trials using prebiotics, NDOs or fibres with diverse objectives that utilised high-throughput approaches (16S rRNA amplicon sequencing, phylogenetic microarrays and metagenomic sequencing), as well as other 16S rRNA-based methods (such as quantitative PCR). Searches were performed to identify human adult and children interventions whereby the effect of inulin-type fructans and other specific NDOs and dietary fibres on the colonic/faecal microbiota were investigated. Initially, a brief description of the 16S rRNA technologies and the key initiatives on human microbiome research are outlined. The specific fibres, NDOs and prebiotics included in this review are then introduced. This is followed by a comprehensive description of the key findings on the effects of these fibres on the faecal microbiota, as well as findings on other physiological parameters. The review concludes with perspectives on future research in this field, which may ultimately lead to strategies on how to obtain hard endpoints for fibres and prebiotics and to aid development of personalised nutrition approaches for specific microbiota and health effects (Figure 1).

2. Technologies and initiatives for human gut microbiome

Many of the early findings on the effects of prebiotics on gut microbiota were incomplete as a number of gut microbial species could not, and still cannot be cultured outside of the host. Despite improvements in the cultivation methods in recent years (Lagier *et al.*, 2016; Lagkouvardos *et al.*, 2017), the more laborious nature, especially of anaerobic culturing, still gives the preference

Figure 1. Scheme for investigating the impact of prebiotics and dietary fibres on the human gut microbiome in human interventions and linking changes to health status for future application such as in personalised nutrition.

to culture independent, 16S rRNA-based technologies (Hiergeist *et al.*, 2015). Analyses of the highly conserved bacterial 16S rRNA gene present in all bacteria gives an accurate estimation of the relative composition of complex microbial communities. Comparison of the 16S rRNA gene in approaches, such as quantitative (real time) PCR, fluorescent *in situ* hybridisation (FISH), and PCR-denaturing gradient gel electrophoresis (TGGE/DGGE) became widely employed in the late 1990's (reviewed by Fraher *et al.*, 2012). Supplementary Table S1 provides a brief summary of the earlier cultivation-independent and -dependent methods.

An overview of the benefits and limitations of the key high-throughput cultivation independent techniques available in gut microbial ecology are presented in Table 1. The high-throughput methods developed in the last two decades include a variety of Next Generation Sequencing (NGS) techniques and phylogenetic microarrays. The microarrays, such as the Human Intestinal Tract Chip (HITChip™) are based on a set of fluorescently labelled oligonucleotide probes that hybridise with complementary nucleotide sequences. The HITChip™ method is highly reproducible and fast, although the community assessment is limited by probe composition. The NGS methods offer parallel sequencing of PCR amplicons of the 16S rRNA gene, or fragmented total (meta)genomic DNA from the whole community (metagenomics/shotgun sequencing), or cDNA reverse transcribed from RNA (metatranscriptomics). A number of different second and third generation sequencing technologies can be used with sequencing taking place on beads (454 Pyrosequencing®, Ion Torrent), slides (Illumina®), wells (PacBio), solid surface (SOLiD™; nanopores (MinION/PromethION) or by electron microscopy (see review of Kumar *et al.*, 2019).

Full understanding of the gut ecosystem requires a comprehensive view into microbiota structure, as well as knowledge on the microbiota activity and finally its functionality (Heintz-Buschart and Wilmes, 2018; Vandeputte *et al.*, 2017). Thus, metagenomics with other 'omics' approaches such as proteomics and metabolomics, as well as their integrated form (meta-omics) can be used together with microbiota composition data to study activity. With any of these methods an enormous amount of information can be gathered even from a single experiment and proper data handling through extensive bioinformatics is essential (Kim and Tagkopoulos, 2018).

Table 1. High-throughput gut microbiota analyses technologies (Fraher *et al.*, 2012; Kumar *et al.*, 2019; Sekirov *et al.*, 2010).

Technique (Platform)	Advantages/disadvantages
16S rRNA gene sequencing or whole genome based	
Second generation sequencing	Short read platforms (35-600 base-pairs (bp)).
Pyrosequencing (454; Roche)	Phylogenetic identification (very) good, PCR bias, labourious, extensive bioinformatics required. No supplier support since 2016, costly.
Synthesis sequencing (Illumina MiSeq, MiniSeq, NextSeq, iSeq HiSeq, NovaSeq)	Phylogenetic identification (very) good, lower error rate than 454, longer run time, high output (1.2-6,000 Gb), PCR bias, laborious. Wide range of sequencing applications possible including amplicon sequencing, metagenome and metatranscriptome. Extensive bioinformatics required, cost effective.
Ion semiconductor sequencing (Ion Torrent)	Read lengths of 200 to 600 bp, output up to 50 Gb, relatively low cost per base, relatively low throughput.
Nanoball Sequencing (BGISEQ-500, MGISEQ-T7)	Similar to Illumina sequencing, low reagent consumption and low cost.
Third generation sequencing	Real time, long read platforms (>1 kb).
Synthesis sequencing (PacBio (currently owned by Illumina) SMRT: RSII, SEQUEL)	Phylogenetic identification good, ultra-long read lengths, single molecule real time sequencing, and good resolution of repetitive regions. High error rates (up to 13%), fast throughput; high cost per base.
Nanopore (MinION, GridION X5 and PromethION)	Highly portable to use in the field, very long reads (>2 Mb), label free, no amplification needed. High error rates (~15%).
Other technologies	
Microarrays (e.g. Human Intestinal Tract-HITChip, Intestinal (I) chip, microbiota array)	Phylogenetic identification very good; semi-quantitative, fast, and easy to use. Phylogenetic identification possible, but novel groups not detected (no sequence on chip). Cross hybridisation, PCR bias, species present in low levels can be difficult to detect; costly.
Non-DNA based	
Metabolomics	Metabolic profiles for comparing communities functionally. More direct functional information on activity. No taxonomic information.
Metaproteomics	More direct functional information. No taxonomic information, less abundant proteins escape detection.
Metatranscriptomics	Insights in community-wide structure and function. Changes in gene expression can be detected. RNA is much less stable than DNA.

The growing realisation of the profound impact of the human microbiota on health, together with the developments in technologies have led to the establishment of numerous large-scale human microbiome projects, and characterisation of microorganisms associated with human health and disease (Hadrich, 2018) (Table 2). Key initiatives included the Human Microbiome Project (HMP) Consortium, which among other body sites, studied the microbiome of faecal samples (Human Microbiome Project, 2012a,b). In Europe the MetaHIT project sequenced the human gut microbiomes from 124 European individuals showing that the total microbial gene set is about 150 times larger than our own human genome (Qin *et al.*, 2010). The International Human Microbiome Consortium that was launched in October 2008 was established to coordinate the microbiome initiatives around the world. A more recent initiative is the American Gut project which is currently the largest, open source and crowd funded microbiome project in the world; the data are for the good of understanding and are shared both with participants and other scientists (Debelius *et al.*, 2016). Still more recent efforts have used computational metagenome binning approach to give metagenome-assembled genomes to capture 1000s of uncultured gut species (Almeida *et al.*, 2019). Microbiome studies continually generate new information: in one recent study using HITChip data with more than 1000 individuals, eight abundant genera were identified in the core microbiome shared by all, namely *Bacteroides, Eubacterium, Faecalibacterium, Alistipes, Ruminococcus, Clostridium, Roseburia* and *Blautia* (Shetty *et al.*, 2017). Another recent study used a compilation of highly curated data from large scale projects to establish a reference database of healthy human gut microbiota and its correlations to different parameters in the metadata, including dietary data. The authors concluded that a healthy human gut is colonised by 8 phyla, 18 families, 23 classes, 38 orders, 59 genera and 109 species, of which 84 species could be referred as core and found in all sampled individuals (King *et al.*, 2019). Thus, the knowledge generated by these initiatives and other research create an essential framework for understanding of the impact of specific fibres and prebiotics on the human gut ecosystem and ultimately will be linked to human health.

3. Fructans, prebiotics and specific dietary fibres

Topline information of the specific prebiotics, NDOs and fibres included in this review are described below and in Table 3, with specific emphasis on inulin. Inulin is a generic term that covers all β-(2,1)-linked linear fructans with a variable degree of polymerisation (DP) and mostly one terminal glucose-unit. Although inulin-type fructans occur in a large variety of plants (Ritsema and Smeekens, 2003; Van Loo *et al.*, 1995), chicory roots (*Cichorium intybus* L.) are the main source for industrial production. Chicory inulin and fructooligosaccharides (FOS), or chicory root fibre, is highly versatile due to the different chain lengths that are manufactured. Chicory inulin, FOS and mixes thereof are applied to a broad range of food applications from fibre or prebiotic enrichment to sugar and fat replacement, or texturizing purposes (Meyer *et al.*, 2007, 2011; Schaafsma and Slavin, 2014). FOS with short chain length (scFOS) may also be synthesised from sucrose (Hirayama and Hidaka, 1993).

Table 2. Various gut microbiome projects around the globe (Stulberg *et al.*, 2016).

Title / initiative	Country	Reference / website
American Gut Project	USA	http://humanfoodproject.com/americangut
British Gut	UK	http://britishgut.org
Canadian Microbiome	Canada	http://www.cihr-irsc.gc.ca/e/39951.html
Elderly gut metagenomics project, ELDERMET	Ireland	http://eldermet.ucc.ie/
Flemish Gut Flora project	Belgium	(Falony *et al.*, 2016)
Human Gut Microbiome and Infections	China	https://www.nature.com/articles/nature11450
Human Microbiome Consortium	USA – Global	http://hmpdacc.org
Human Metagenome Consortium	Japan	http://www.jchm.jp
International Human Microbiome Consortium (IHMC)	International	http://www.human-microbiome.org
Metagenomics of the Human Intestinal Tract (MetaHIT)	Europe	http://www.metahit.eu
MicroObes, Human Intestinal Microbiome in Obesity and Nutritional Transition	France	http://www.inra.fr/micro_obes_eng
Korean Microbiome Diversity using Korean Twin Cohort Project	Korea	(Lim *et al.*, 2014)
LifeLines	The Netherlands	https://lifelines.nl/home
MetaGenoPolis	France	http://www.mgps.eu/index.php?id=accueil
Michigan Microbiome Project	USA	http://microbe.med.umich.edu/about/research/michigan-microbiome-project
The Australian Jumpstart Human Microbiome Project	Australia	http://www.human-microbiome.org/index.php?id=30#c77

Table 3. Description of inulin-type fructans, other non-digestible oligosaccharides and dietary fibres included in this review.[1]

Fibre / prebiotic	Description	References
β-fructans / inulin-type fructans		
Agave fructans	Extracted from agave, DP up to about 30; branched and highly branched fructans in 1:4.1 ratio	Carranza *et al.*, 2015
Chicory native inulin	Inulin as extracted from chicory with DP 2-60	Mensink *et al.*, 2015
Chicory FOS	Produced from inulin by partial enzymatic hydrolysis, DP 2-8	
Chicory lc-inulin/FOS	Long chain inulin produced from native chicory inulin, DP 10-60	
Chicory FOS/oligofructose		
Chicory sc-inulin	Short chain inulin produced from native chicory inulin, DP 2-10	
scFOS	Produced enzymatically from sucrose, DP 2-5	Hirayama and Hidaka, 1993
Jerusalem artichoke inulin	Extracted from Jerusalem artichoke, DP range 2 to >30	Saengthongpinit and Sajjaanantakul, 2005
Other non-digestible oligosaccharides		
AXOS	Isolated from wheat bran; xylan chains with a variable substitution level of arabinose side chains	Swennen *et al.*, 2006
(sc)GOS / TOS	Produced from lactose by β-galactosidase; β-1,6-linked galactosyl residues (DP 2-5) linked to terminal glucose unit via β-1,4-bond	Coulier *et al.*, 2009
β-GOS	Produced with a novel *Bifidobacterium* β-galactosidase enzyme	Depeint *et al.*, 2008
XOS	Produced by partial enzymatic hydrolysis of xylan from birch wood; mixture of xylose and XOS, mostly DP 2-3	Aachary and Prapulla, 2010
Other dietary fibres		
Arabic gum/ acacia gum	Produced from hardened sap of acacia tree; mixture of glycoproteins and polysaccharides, mainly arabinose and galactose	Williams and Phillips, 2009
Konjac glucomannan	Extracted from konjac tubers; chain of D-mannose and D-glucose with a α-1,4-pyranoside bond and a few acetyl groups at C-6 position of the side chain	Yang *et al.*, 2017
Beta-glucans	Glucose molecules in long linear polymers with blocks of 2-4 glucose units linked by β-(1→4) (70%), and separated by β-(1→3) glucose links (30%)	Wood, 2007
PHGG	Isolated from guar seeds and partial enzymatic hydrolysis	Mudgil *et al.*, 2014
PDX	Prepared by thermal polymerization of glucose; contains various types of glycosidic bonds	Craig *et al.*, 1998
Resistant starches		
RS1	Physically inaccessible or digestible resistant starch from e.g. seeds, legumes, unprocessed whole grain	Homayouni *et al.*, 2014
RS2	Natural granular form (e.g. from uncooked potatoes)	
RS3	Retrograded starch like cooked & cooled starchy foods	
RS4 e.g. SCF (RM)	Produced from wheat or corn starch by controlled dextrinization to DP 10-30 with 1,2 and 1,3 glycosidic linkages; or produced by combination of heat and enzymatic treatment; wide variety of structures not found in nature. May be mixtures of α(1→6), α(1→4), α(1→2), and α(1→3) glucosidic linkages	

[1] DP = degree of polymerisation; FOS = fructooligosaccharides; AXOS = arabinoxylooligosaccharides; GOS/TOS = (trans)-galactooligosaccharides; lc = long chain; PDX = polydextrose; PHGG = Partially hydrolysed guar gum; RM = resistant maltodextrin; RS = resistant starch; sc = short chain; SCF = soluble corn fibre; XOS = xylooligosaccharides.

Several other NDOs besides chicory FOS and scFOS are included in this review: the prebiotic galactooligosaccharides (GOS) produced on an industrial scale from milk lactose, which usually contain lactose, glucose and galactose (Torres *et al.*, 2010); xylooligosaccharides (XOS); and arabinoxylooligosaccharides (AXOS) which are not (yet) commercially available. A 9:1 combination of GOS/ long chain inulin is widely used in infant and follow-on formulae for its prebiotic effect (Knol *et al.*, 2005; Rinne *et al.*, 2005; Scholtens *et al.*, 2006). Well known dietary fibres included in this review are Arabic gum, beta-glucans, konjac glucomannan, partially hydrolysed guar gum (PHGG) and polydextrose (PDX) synthesised from glucose often of corn origin (Table 3). Commercially available resistant starches (RS), including soluble corn fibre (SCF), that exhibit dietary fibre features are also included.

Literature searches were performed using string searches with a combination of terms for dietary fibres and prebiotics with 16S rRNA-based approaches to study human gut microbiota in the PubMed Central database (until 10 September 2019) (Supplementary Table S2). Relevant extra studies were retrieved by input from experts in the field from sources such as internal company/university databases and reading the publications found. All abstracts were read to identify relevant full publications. This is a qualitative review; the criteria for inclusion of the 16S rRNA sequencing and phylogenetic array approaches were: (1) human clinical studies with single defined dietary fibres or prebiotics, or defined mixes with specified dose; (2) (randomised) human trials, blinded (unless explanation provided), at least one week duration; (3) inclusion of a placebo or control group; and (4) healthy children (not infants), adults and elderly subjects. In addition, the overweight/ obese subjects, and subjects that had various disease states such as Crohn's disease, irritable bowel syndrome (IBS), type 2 diabetes, amongst others, were also included. Studies were included whether the microbiota analysis was primary or secondary. The key outcome reported was for treatment (fibres, NDOs, prebiotics) versus placebo/control, with respect to relative abundance or counts of bacterial groups depending on the molecular technique. The impact of baseline levels of gut bacteria on outcome were discussed only secondarily, and when treatment versus placebo comparison was not available. The outcome for community-wide molecular technologies, i.e. sequencing and microarrays, which were the focus of this review are presented in Tables 4, 5 and 6, while other molecular technologies are presented in the Supplementary Tables S3, S4 and S5.

4. Impact of prebiotics and specific fibres on the human gut microbiota

Inulin-type fructans

A total of seventeen human studies to date used high-throughput 16S rRNA-based technologies to investigate effects of inulin-type fructans on gut microbiota (Table 4). Fifteen studies used 16S rRNA gene sequencing, twelve of these studies applied the Illumina MiSeq platform, one used Ion Torrent platform, and another two studies used microarrays. These human interventions, which described the effects of mainly chicory inulin-type fructans on the colonic microbiota, confirmed the selective bifidogenic effect reported in the earlier culture-based studies, while giving less impact on other gut microbial species (Table 4).

The studies using high-throughput sequencing or microarray technologies are mainly discussed below in more detail. Many of these studies simultaneously investigated the impact on parameters of digestive or gut health. The application of the HITChip microarray enabled a comprehensive qualitative view into the effects of inulin-type fructans on a wide range of faecal microbiota. A HITChip based study on the effect of chicory FOS consumption (20 g/d) in healthy volunteers reported an increase in the relative abundance of *Bifidobacterium* and a concurrent decrease of *Bacteroidetes*; there was no effect on alpha -diversity in this study (Tims *et al.*, 2016). In the latter high dose chicory FOS study, fermentation increased faecal wet weight and mucin excretion (Ten Bruggencate *et al.*, 2006). Interestingly, a shift in interactions of acetate- and lactate-utilising and butyrate-producing genera, monitored using the butyryl-CoA:acetate CoA transferase gene of the faecal ecosystem was observed for chicory FOS. This novel analysis gave insight in the cross-feeding and butyrate effect of fructans in the human colon, i.e. that inulin fermentation was associated with concurrent increase in butyrate levels, despite the fact that bifidobacteria derived fermentation products comprise acetate and lactate only. The intake of 5 and 7.5 g/d agave fructans improved bowel habit parameters in the study subjects and increased relative abundance of faecal *Actinobacteria* and specifically *Bifidobacterium* as shown by 16S rRNA amplicon sequencing (Holscher *et al.*, 2015a). Percent of sequencing reads belonging to *Bifidobacterium* in control subjects in this study was 1.7%, while in subjects consuming 5 and 7.5 g/d agave inulin it was 3.2 and 4.9%, respectively. Furthermore, *Faecalibacterium* showed a positive correlation with faecal butyrate concentration, while bifidobacteria correlated negatively with levels of faecal ammonia (Holscher *et al.*, 2015a). In a study investigating butyrate production, subjects consumed 20 g/d chicory inulin for 2 weeks slowly increasing the dose during the first week; this significantly increased relative abundance of various *Bifidobacterium* species and *Anaerostipes hadrus,* although it did not increase faecal butyrate levels in this short term trial, relative to resistant starch, as described below (Baxter *et al.*, 2019). In a study with 12 g/d of native chicory inulin studying impact on stool frequency in constipated subjects, increased relative abundance of *Bifidobacterium* and *Anaerostipes* spp. and a decreased relative abundance of *Bilophila* spp. were noted in the constipated subjects, as well as an improved stool frequency (Micka *et al.*, 2017; Vandeputte *et al.*, 2017). Another two joint studies investigating bowel habits with 10 g/d native chicory inulin in healthy adults showed no significant differences in specific bacterial abundance nor alpha-diversity, although trends in similar directions to other studies were noted (Watson *et al.*, 2019). Nevertheless, there was improved stool frequency when subjects had low stool frequency at baseline, and softer stool consistency reported for the treatment compared to placebo. In another study, 16 g/d of chicory FOS was studied for its effect in subjects with orlistat-induced fat malabsorption which increases fat in the colon of the volunteers; relative abundance of *Bifidobacterium* sp. was increased and there were no other changes in global microbiota composition (Morales *et al.*, 2016). The negative effects of the orlistat tended to be averted by FOS which prevented the increase of faecal calprotectin suggesting it could be beneficial in reducing colonic inflammation.

Table 4. Effect of inulin-type fructans (ITF) on human gut microbiota composition.[1]

ITF	Dose g/d	Subjects	Trial design; duration	Technology	Outcome (versus control)	Reference
Agave inulin in chocolate chews	5 or 7.5	Healthy adults (n=29)	RCT, DB, three period CO, placebo-chocolate chews with no supplementation; 21 d, 7 d WO	16S rRNA sequencing (Illumina Miseq)	*Actinobacteria, Bifidobacterium* ↑ 3- and 4-fold after 5.0 and 7.5 g; *Lachnobacterium, Ruminococcus*, *Desulfovibrio* ↓	Holscher *et al.*, 2015a
Chicory FOS	20	Healthy volunteers (n=28)	RCT, DB, CO, placebo sucrose; 2 wk intervention, 1 wk WO in between	HITChip microarray	*Bifidobacterium* ↑; *Bacteroidetes* ↓; no effect on diversity	Tims *et al.*, 2016
Chicory FOS	16	Healthy volunteers (n=41)	RCT placebo-maltodextrin; 1 wk baseline, 1wk WO, 1 wk intervention, 1 wk follow-up	16S rRNA sequencing (Illumina Miseq), RT-PCR for *Bifidobacterium* and *Lactobacillus*	*Bifidobacterium* ↑; alpha-diversity not changed.	Morales *et al.*, 2016
Chicory FOS supplement in low FODMAP diet	14	Healthy adults (n=37)	RCT, P, placebo-maltodextrin with pre and post diet assessment; 1 wk, 1 wk run-in period	16S rRNA sequencing (Illumina Miseq)	Bifidobacteria ↓ in low FODMAP diet; *Ruminococcaceae* ↑ in placebo group; Bifidobacteria ↑, *Lachnospiraceae* ↓ in FOS group	Sloan *et al.*, 2018
Chicory inulin	12	Healthy volunteers (n=44)	DB, RCT, CO placebo-maltodextrin; 4 wk	16S rRNA sequencing (Illumina Miseq)	*Bifidobacterium* ↑, *Anaerostipes* ↑; *Bilophila* ↓; no effect on other genera; diversity ↓	Vandeputte *et al.*, 2017
Chicory inulin	8	Type 1 diabetic children (n=43)	RCT, DB, P, placebo maltodextrin; 3 mo, 3 mo WO at end	16S rRNA sequencing (Illumina Miseq), qPCR for bifidobacteria	*Bifidobacterium* ↑ (*B. longum*), *Coriobacteriales* ↑ also versus baseline; *Bifidobacterium* ↓ after 6 mo. alpha-diversity slightly ↓	Ho *et al.*, 2019
Chicory inulin (or RS2)	20	Healthy young (17-29 yr) adults (n=174)	RCT, P, control accessible corn starch; 2 wk over yrs	16S rRNA sequencing (Illumina Miseq)	*Bifidobacterium, Anaerostipes hadrus* ↑	Baxter *et al.*, 2019
Chicory inulin	2×5	Middle-age to older adults (40-75 yr): Trial A (n=10), Trial B (n=20)	RCT, CO, placebo-maltodextrin; 5 wk, 2 wk WO	16S rRNA sequencing (Illumina MiSeq)	No significant changes nor in alpha-diversity	Watson *et al.*, 2019
Chicory FOS:lc-inulin (1:1) powder in drinks	2×8	Obese females (n=15)	RCT, DB, placebo-maltodextrin; 3 mo	HITChip microarray, qPCR for *Bifidobacterium* spp., *Lactobacillus* spp., *Lactobacillus acidophilus*	*Bifidobacterium, Faecalibacterium prausnitzii* ↑, *Bacteroides, Propionibacterium* ↑; qPCR: Bifidobacteria, lactobacilli ↑	Dewulf *et al.*, 2013
Chicory FOS:inulin in bar	6 + 2	Healthy adults with overweight/ obesity (n=25)	RCT, DB, P; placebo-control bar; 12 wk	16S rRNA sequencing (Illumina Miseq)	*Bifidobacterium* ↑, alpha-diversity ↓	Reimer *et al.*, 2017
Chicory FOS:lc-inulin mix	16	Healthy, low (LDF) or high dietary fibre (HDF) consumers (n=34)	RCT, DB, CO; placebo-maltodextrin; 2× 3 wk & 3 wk WO	16S rRNA sequencing (Illumina Miseq)	LDF: *Bifidobacterium* ↑, *Lactobacillus* ↑, unknown genus of *Ruminococcaceae* ↓. HDF: *Bifidobacterium* ↑, unknown genus in *Ruminococcaceae* ↑, *Faecalibacterium, Coprococcus*, *Dorea* and *Ruminococcus* ↓ (*Lachnospiraceae* family)	Healey *et al.*, 2018

Table 4. Continued.

ITF	Dose g/d	Subjects	Trial design; duration	Technology	Outcome (versus control)	Reference
Chicory FOS:lc-inulin (1:1)	8	Healthy overweight or obese children (n=22)	Single-centre, DB, placebo maltodextrin; 16 wk	RT-PCR and 16S rRNA sequencing (Illumina Miseq)	*Bifidobacterium* ↑, *Bacteroides vulgatus* ↓ *Clostridium* cluster XI ↓, *F. prausnitzii* ↓	Nicolucci *et al.*, 2017
Chicory lc-inulin	13-15	Overweight men (BMI 25-35 kg/m^2) (n=19)	RCT, CO, DB, isoenergetic control diet; 21 d, 21 d WO	16S rRNA sequencing (Illumina MiSeq)	*Bifidobacterium* ↑	Blædel *et al.*, 2016
Chicory sc-inulin & lc-inulin mix	6	3-6 y healthy & antibiotic-treated children (n=258)	RCT, DB, placebo-maltodextrin; 24 wk	16S rRNA sequencing (Illumina multiplex); qPCR for total bacteria, *Bifidobacterium, Lactobacillus, C. difficile, C. perfringens,* and *Enterobacteriaceae*	qPCR: *Bifidobacterium* ↑, *Lactobacillus* ↑; sequencing: bifidobacteria ↑, also in antibiotic-treated children; no other differences reported	Lohner *et al.*, 2018; Wang *et al.*, 2016; Soldi *et al.*, 2019
Chicory sc-inulin OR sc-inulin+probiotic mix in capsules	2×6	Asthmatic adults (n=17)	RCT, DB, CO; placebo-cellulose; 7 d	16S rRNA sequencing (not specified)	sc-inulin: *Anaerostipes* ↑, *Erysipelotrichaceae*↓; trend *Bifidobacterium* ↑ (significant only from baseline); sc-inulin+probiotic: *Bifidobacterium* ↑	McLoughlin *et al.*, 2019
scFOS versus GOS	16	Healthy adults (n=35)	RCT, DB, CO; 1 wk run in period, 14 d	16S rRNA sequencing (Ion Torrent)	FOS, GOS: *Bifidobacterium* ↑; FOS: *Phascolarctobacterium* ↓; GOS: *Ruminococcus* ↓	Liu *et al.*, 2017a
scFOS	2.5, 5, 10	Healthy adults (n=80, 20 per arm)	RCT, DB, placebo 10 g maltodextrin; 60 d run-in, 90 d intervention, 60 d follow-up phase	16S rRNA sequencing (Illumina Miseq)	*Bifidobacterium* ↑ strongly & *Lactobacillus* ↑ especially for 10 g dose; *Faecalibacterium, Ruminococcus* & *Oscillospira* ↑. Upon discontinuation FOS *Bifidobacterium* ↓, *Oscillospira* ↑.	Tandon *et al.*, 2019

[1] BMI = body mass index; CO = crossover; DB = double blind; FOS = fructooligosaccharides; GOS = galactooligosaccharides; lc = long chain; P = parallel, qPCR = quantitative PCR; RCT = randomised controlled trial; RT-PCR = real time PCR; sc = short chain; WO = washout; ↑↓, significantly increased or decreased.

Several other interventions for fructans that monitored gut microbiota with high-throughput technologies simultaneously studied the impact on weight management or metabolic syndrome parameters. The gut microbiota of obese women was monitored using the HITChip following consumption of 16 g/d mixture of chicory FOS and long chain inulin for 3 months (Dewulf *et al.*, 2013). The modifications in the microbiota due to inulin were subtle, still the fructans significantly increased relative abundance of *Firmicutes* (mainly bacilli, and *Clostridium* clusters IV and XVI) and *Actinobacteria*, while there was a decrease in *Bacteroidetes*. The relative abundances of *Bifidobacterium* and *Faecalibacterium prausnitzii* also increased and this negatively correlated with serum lipopolysaccharide levels. There was a decreased relative abundance of *Bacteroides,* specifically *Bacteroides intestinalis* and *Bacteroides vulgatus* and *Propionibacterium* in the inulin group, which positively correlated with changes in body composition and glucose homeostasis; an increased proportion of *Lactobacillus* spp. was also detected using qPCR. In another study, analysis of faecal microbiota using the Illumina platform of overweight/obese adults showed that chicory inulin-type fructans added to snack bars significantly increased relative abundance of bifidobacteria while decreased alpha-diversity; several aspects of appetite control were improved (Reimer *et al.*, 2017). Analysis of microbial functions by PICRUSt (predictive software based on the 16S rRNA composition) on the effects of inulin-type fructans on the microbiota in the latter snack bar study showed that supplementation was associated with changes in community genetic potential related to genetic information processing, metabolism of amino acids, nucleotides, terpenoids, polyketides and other pathways (Reimer *et al.*, 2017). The third intervention studied the effect of consumption of 8 g/d of a 1:1 mix of chicory FOS and long chain-inulin in overweight and obese children, using 16S sequencing and a battery of qPCR probes (Nicolucci *et al.*, 2017). There was a significant increase in proportion of *Bifidobacterium* spp. while there was a decrease in *B. vulgatus*. Consumption of this mix was associated with significant reduction of body fat and body weight, significant decrease in serum triglycerides and serum level of interleukin 6 in these children. Another intervention with child Type I diabetes patients using the same inulin mix and dose also increased the relative abundance of *Bfidobacteria* at 3 months which was gone after a further 3 month washout; there were no improvements in diabetic ketoacidosis although C-peptide was significantly higher and there was a trend for improved intestinal permeability (Ho *et al.*, 2019). Finally the impact of 16 g/d scFOS or GOS on glycemia during oral glucose tolerance test (OGTT) and the intestinal microbiota was studied (Liu *et al.*, 2017a). The short-term intake of 14 d, which suggested both prebiotics had an adverse effect on the glucose response, increased relative abundance of *Bifidobacterium,* and reduced the relative abundance of *Phascolarctobacterium, Enterobacter, Turicibacter, Coprococcus* and *Salmonella* in the FOS group.

Supplementation of 12 g/d of sc-inulin to asthmatic adults showed increased relative abundance of *Anaerostipes* and a trend for that of *Bifidobacterium,* while *Roseburia* and *Erysipelotrichaceae* relative abundances decreased (McLoughlin *et al.*, 2019). Post-hoc group analyses showed an improvement in asthma control and airway inflammation for the sc-inulin treatment compared to control and to a synbiotic treatment, and the changes in *Anaerostipes* and *Roseburia* were associated with these effects. Another intervention with fructans investigated the effect of a 1:1 mix of chicory FOS and long chain inulin in healthy middle-aged subjects on response to influenza vaccine and on gut microbiota composition (Lomax *et al.*, 2012). A bifidogenic effect occurred with no effect on the counts of total bacteria as shown by FISH, and there was no improvement in vaccine response. In a follow-up study, some aspects of the antibody response to vaccination were improved (Lomax *et al.*, 2015). Recently, chain length-dependent effects of inulin-type fructans on the human systemic immune responses have been discovered, such that the long chain inulin could stimulate antibody responses in humans to a vaccine, in contrast to short chain inulin; it was hypothesised that the long chained fructan may interact with receptors in the small intestine (Vogt *et al.*, 2017). Thus, specific chain lengths of fructans might be required for specific immune effects, and these effects might not be necessarily microbiota-mediated.

Human studies utilising earlier 16S rRNA-based methods (FISH, qPCR, band sequencing from PCR-DGGE and TRFLP) showed that fructans from sources such as chicory roots, Jerusalem or globe artichoke and agave in various food matrices generally resulted in increased levels of bifidobacteria in faeces of adults and children (Supplementary Table S3). Relatively low doses from 2.5 gram/day (g/d) could achieve this bifidogenic effect. Only two studies using FISH or qPCR showed no effect on faecal levels of bifidobacteria: one FISH study using a 15 g/d mix of chicory FOS and long chain inulin compared to maltodextrin placebo in Crohn's disease patients (Benjamin *et al.*, 2011), and the second study, which used 20 g/d of a similar FOS:inulin mix in comparison to lactulose, which is also known to have a bifidogenic effect (De Preter *et al.*, 2008).

A few studies looked more specifically into the species and strain level responses due to fructan supplementation, a majority using low throughput methods alone (Table S3 or in combination with high-throughput methods as in Table 4). These studies confirmed that consumption of chicory fructans can be associated with increased proportions of species *Bifidobacterium bifidum*, *Bifidobacterium adolescentis, F. prausnitzii* (Joossens *et al.*, 2011; Ramirez-

Farias *et al.*, 2009), and *Bifidobacterium longum* in stools (Joossens *et al.*, 2011). A study with agave fructans showed a significant increase specifically in *B. adolescentis, B. longum, B. bifidum* (Nicolucci *et al.*, 2017), *Bifidobacterium breve,* and *Bifidobacterium pseudolongum* (Holscher *et al.*, 2015a). In a follow-up of one human study, analysis of butyryl-CoA:acetate CoA-transferase sequences using degenerate primers confirmed the increased relative abundance for *F. prausnitzii* for 10 g of chicory inulin (Louis *et al.*, 2010; Ramirez-Farias *et al.*, 2009).

One study concluded that the microbial and metabolic responses can vary between subjects due to differences in the initial colonic conditions including baseline levels, and metabolic activity of microbiota (bifidobacteria) (De Preter *et al.*, 2008). Investigation of the influence of the baseline bifidobacteria levels by qPCR showed a significant correlation between baseline counts in the stools and the effect of the inulin intake, indicating that the baseline bifidobacteria levels affects the magnitude of the bifidogenic response. Habitually high dietary fibre intake was associated with stronger responses in microbiota to chicory FOS:inulin mix supplementation as suggested in one study (Healey *et al.*, 2018). Significant increase in proportions of *Faecalibacterium* and decreases in *Coprococcus, Dorea* and *Ruminococcus* (*Lachnospiraceae* family) were noted in subjects habitually consuming high fibre diets, however, the study was limited by uneven distribution of participants in the low/high habitual fibre study groups (Healey *et al.*, 2018).

In conclusion, studies on the impact of inulin-type fructans on the human gut microbiota using advanced 16S rRNA-sequencing technologies are dominated by chicory inulin-type fructans and limited for other fructan types (Table 4). The majority of studies showed a bifidogenic effect for chicory inulin-type fructans, notably for *B. adolescentis.* In some studies, the relative abundances of *Anaerostipes* and lactobacilli also increased, *F. prausnitzii* had variable response, and sometimes the relative abundances of *Bacteroidetes, Bilophila* and *Ruminococcus* decreased. The few studies with FOS, agave and Jerusalem artichoke fructans showed increased relative abundance of bifidobacteria and sometimes lactobacilli, while sometimes the relative abundances of various other groups/ genera were reduced. Other changes in the gut microbiota were often more subtle and variable. Inulin consumption resulted in a strong (Reimer *et al.*, 2017), modest (Vandeputte *et al.*, 2017) or no effect (Bogovic Matijasic *et al.*, 2016; Nicolucci *et al.*, 2017) on global microbiota composition (alpha-diversity).

Further studies are required to investigate the effect of different fructan types, fructan mixes, doses and different chain lengths on the human gut microbiota, microbial diversity and global community structure. High inter-individual variation in gut microbiota between human study subjects was also proposed as a factor that may influence the outcome of fibre effects on gut microbiota (Morales *et al.*, 2016). As these factors are important for host intestinal and overall health, there is a need to simultaneously study broader microbial changes in response to inulin-type fructans. In addition, the effects of fructans on the different regions of the human intestinal tract should be examined in future studies.

GOS, AXOS and XOS

The studies investigating GOS, XOS and AXOS are reported in Table 5 and Supplementary Table S4. A total of eight human studies using high-throughput 16S rRNA-based technologies, including two with phylochips, investigated effect of GOS on human gut microbiota (Table 5). Initially 16S sequencing was used to gain a community wide perspective of the impact of increasing doses (2.5, 5, and 10 g/d) of GOS on the faecal microbiota of healthy subjects for 14 weeks (Davis *et al.*, 2011); GOS led to five- to ten-fold increases in bifidobacteria in half of the subjects and the effect was dose-dependent. Another study that used pyrosequencing and 16S rRNA species-specific primers showed that supplementation with a highly purified GOS was also associated with higher relative abundance of *Bifidobacterium* (average relative abundance increased from 0.001 at day (d) 0 to 0.007 at d 36), specifically of *B. adolescentis* (45 to 8,212-fold increase), *B. longum* (42 to 108-fold increase), *Bifidobacterium catenulatum* (25 to 1,874-fold increase), *B. breve* (average 46-fold increase as detected by the GroEL probe), *Bifidobacterium dentium* (Azcarate-Peril *et al.*, 2017) and other *Bifidobacterium* operational taxonomic unit phylotypes. Bifidobacteria effects were dose-dependent with no effect at 2.5 but increasing effect at 5 and 10 g/d (Davis *et al.*, 2011). In the latter two studies, *F. prausnitzii* was stimulated by GOS intake at 5 and 15 g/d which was also occasionally observed for inulin-type fructans (Azcarate-Peril *et al.*, 2017; Davis *et al.*, 2011); additionally, the relative abundances of *Lactobacillus*, an unidentified genera of the family *Christensenellaceae* (Azcarate-Peril *et al.*, 2017) and *Coprococcus comes* were higher due to GOS intake (Davis *et al.*, 2010, 2011). After the washout period, it was observed that relative abundance of bifidobacteria amongst other groups was reduced, as expected (Azcarate-Peril *et al.*, 2017; Liu *et al.*, 2017a).

A few human studies investigated GOS in terms of gut health aspects. GOS had no synergistic beneficial effect in the synbiotic application with the probiotic strains *B. adolescentis* IVS-1 and *Bifidobacterium lactis* BB-12 (Krumbeck *et al.*, 2018); the relative abundance of *Lachnobacterium* decreased upon GOS intervention (Krumbeck *et al.*, 2018). GOS showed a beneficial effect on recovery of the intestinal bifidobacteria though not

Table 5. Effect of arabinoxylan-oligosaccharides (AXOS), galactooligosaccharides (GOS) and xylooligosaccharides (XOS) on human gut microbiota composition.[1]

Type	Dose g/d	Subjects	Trial design and duration	Technology	Outcome (versus control)	Reference
AXOS	10.4	Overweight, obese subjects (25-40 kg/m^2) (n=30)	RT, CO, no placebo; 4 wk, 4 k WO 4 wk	Shot-gun sequencing (HiSeq2500 platform)	Compared to baseline only: *Actinobacteria* ↑, *Bifidobacteriaceae* ↑, *Bifidobacterium* ↑, *Ruminococcus gnavus* ↑, *Lachnospiraceae* groups ↑, *Prevotella* ↑, *Rikenella* ↓, *Parabacteroides* ↓, *Paraprevotella* ↓ species	Benítez-Páez *et al.*, 2019
GOS (75% w/w; 59% GOS; 21% lac, 19% glu, 1% gal)	2.5×3 (7.5)	Healthy volunteers (n=12)	RCT, DB, P placebo-maltodextrin 12 d total: 5 d AMX + GOS (n=6) / placebo (n=6), followed by 7 d no AMX	Intestinal-chip/ microarray, qPCR for total bacteria and *Bifidobacterium* spp.	GOS with antibiotic treatment ↑ bifidobacteria; faster recovery to normal with GOS	Ladirat *et al.*, 2014
GOS (69%; 23% lac, 22.8% lac, 4.7% glu)	15	Overweight or obese, pre-diabetic (n=44)	RCT, DB, P; 12 wk; placebo-maltodextrin	HITChip microarray	*Bifidobacterium* ↑	Canfora *et al.*, 2017
GOS (72,5%; 23% lac, 5% glu + gal), GOS+*Bifidobacterium adolescentis* IVS-1 + *Bifidobacterium lactis* BB-12	5	Obese adults (n=114)	RCT, DB, P, placebo-lactose; 3 wk	16S rRNA sequencing (Illumina Miseq); qPCR for *Bifidobacterium, B. adolescentis*	*Bifidobacterium* ↑ in GOS: IVS-1 induced higher levels of bifidobacteria than Bb12; no functional synergism when used as synbiotic	Krumbeck *et al.*, 2018
GOS (80%)	5.5	T2D men (n=29)	RCT, P; placebo-maltodextrin; 12 wk	16S rRNA sequencing (GS FLX Titanium platform), qPCR for total bacteria, *Bifidobacterium, Roseburia, Lactobacillus, Enterobacteriaceae, Clostridium leptum, Clostridium coccoides* groups	No significant changes on bacterial abundances compared with placebo; trend ↑ *Bifidobacterium*	Pedersen *et al.*, 2016
GOS (80%) in gluten and casein-free diets, and in unrestricted diet	1.44	Autistic children (4-11 y) (n=30)	RCT, DB, P, placebo-maltodextrin; 6 wk	16S rRNA sequencing (Illumina Miseq); FISH for total bacteria and *Bifidobacterium* spp.	*Bifidobacterium longum* ↑ in exclusion diet + GOS group. Positive association of GOS with *Bifidobacterium* spp., *Ruminococcus* spp., *Lachnospiraceae* family (*Coprococcus* spp., *Dorea formicigenerans, Oribacterium* spp.), *Eubacterium dolchum*, *TM7-3* family and *Mogibacteriaceae*	Grimaldi *et al.*, 2018

Table 5. Continued.

Type	Dose g/d	Subjects	Trial design and duration	Technology	Outcome (versus control)	Reference
GOS (91.8%; 7% lac, 1% glu, 0.5% gal) in caramel chews	2.5, 5.0 or 10	Healthy volunteers (n=18)	Single blinded (randomisation not specified); 2 wk baseline, 3 wk for each dose, no WO between; 2 wk final WO	16S rRNA pyrosequencing (454)	0, 2.5 g dose: no change. 5 & 10 g doses: *Bifidobacteriaceae*, *Bacteroidaceae* ↑; *B. adolescentis, B. longum, Bifidobacterium catenulatum* ↑. At 5 g only: *Faecalibacterium prausnitzii* ↑; At 10 g only: *Coprococcus* ↓	Davis *et al.*, 2011
GOS/RP-G28 (purified scGOS >95%)	15	Lactose-intolerant subjects (n=52)	RCT, DB, P, multi-site; placebo-corn syrup; 30 d	16S rRNA pyrosequencing (454) on treatment & qPCR for *Bifidobacterium* species; T-RFLP on placebo	From baseline bifidobacteria ↑ 27/30 subjects (90%); qPCR: lactose-fermenting *Bifidobacterium, Faecalibacterium* and *Lactobacillus* ↑	Azcarate-Peril *et al.*, 2017
GOS (95% purity) versus scFOS	16	Healthy adults (n=35)	RCT, DB, CO; 1 wk run in period, 14d	16S rRNA sequencing (Ion Torrent)	GOS, FOS: *Bifidobacterium* ↑; GOS: *Ruminococcus* ↓; FOS: *Phascolarctobacterium* ↓	Liu *et al.*, 2017a
XOS in capsules	1.4 or 2.8	Healthy adults (n=32)	RCT, DB, placebo-maltodextrin; 2 wk run in, 8 wk intervention, 2 wk WO	16S rRNA pyrosequencing (GS FLX Titanium); Culturing: *Enterobacteriaceae*, *Bacteroides fragilis* group, *Clostridium, Bifidobacterium, Lactobacillus*	No change alpha-diversity; *Faecalibacterium* and *Akkermansia* ↑ at specific wk; *Bifidobacterium* ↑ on both doses by culturing only at 8 and 10 wk, anaerobic counts ↑ and *B. fragilis* counts ↑ at 2.8 g	Finegold *et al.*, 2014
XOS in capsules	2	Healthy (n=16) and pre-T2D subjects (n=13)	RCT, P, placebo-maltodextrin; 8 wk	16 rRNA sequencing (Illumina MiSeq)	Significant differences in healthy & pre-DM microbiota composition. In pre-T2D: ↓ *Howardella*, *Slackia*, *Enterorhabdus.* No change in *Bifidobacterium* (genus and 3 spp.) in healthy volunteers	Yang *et al.*, 2015

[1] AMX = amoxicillin; AXOS = arabinoxylooligosaccharides; CO = crossover; DB = double blind; DM = Diabetes Mellitis; FISH = fluorescent *in situ* hybridisation; FOS = fructooligosaccharides; gal = galactose; glu = glucose; GOS = galactooligosaccharides; lac = lactose; P = parallel; qPCR = quantitative PCR; RCT = randomised controlled trial; RT-PCR = real time PCR; sc = short-chain; T2D = type 2 diabetes; WO = washout; XOS = xylooligosaccharides; ↑↓ = significantly increased or decreased.

significantly, however there was a significant increase in butyrate levels after antibiotic treatment in healthy adults (Ladirat *et al.*, 2014). A higher dose of highly purified GOS (>95% purity; 15 g/d) consumed by lactose-intolerant individuals significantly shifted subject's microbiota composition, increased abundance of lactose-fermenting *Bifidobacterium, Faecalibacterium,* and *Lactobacillus* and had a positive effect on lactose digestion and tolerance (Azcarate-Peril *et al.*, 2017). In fact, the higher relative abundance of *Bifidobacterium* was negatively correlated with cramping and pain in lactose-intolerant subjects. Furthermore, PICRUSt analysis was included in this study and revealed changes in the community genetic potential with a gradual increase in abundance of predicted enzymes involved in GOS metabolism during GOS intake. In a recent study GOS intervention was positively correlated with improvements in anti-social behaviour in autistic children, and there was significant increase in abundance of *Lachnospiraceae* family; bifidobacteria relative abundance had also increased though not significantly (Grimaldi *et al.*, 2018).

There were three studies investigating GOS intake with overweight, type-2- or pre-diabetic subjects. In a study on GOS effects on microbiota of type 2 diabetic patients, supplementation at 5.5 g/d was not associated with microbial community shifts and the bifidogenic effect did not reach significance in this cohort, possibly due to interaction with the medication (Metformin) and the high heterogeneity of human type 2 diabetes (Pedersen *et al.*, 2016). In addition, there was a negative correlation between abundance of family *Veillonellaceae* after GOS intake. The GOS supplementation had no significant effects on clinical outcomes such as intestinal permeability and glucose tolerance (Pedersen *et al.*, 2016). In a second study, supplementation of diets of obese, pre-diabetic subjects with 15 g/d GOS for 12 weeks increased the abundance of *Bifidobacterium* species in faeces by 5-fold, while microbial richness or diversity were not changed, as measured by HITChip (Canfora *et al.*, 2017); there were no significant changes in insulin sensitivity or related substrate and energy metabolism in the subjects. Finally, short-term intake of 16 g/d GOS increased relative abundance of *Bifidobacterium* and decreased relative abundances of *Ruminococcus, Dehalobacterium, Synergistes* and *Holdemania* some of which are butyrate-producing microbes, and it was speculated that this may have played a role in the adverse effect on glucose metabolism, as measured by fasting glucose levels (Liu *et al.*, 2017a).

Two studies to date utilised 16S rRNA sequencing to measure the microbial effects of XOS. XOS supplementation at 1.4 or 2.8 g/d for 10 weeks showed no notable shifts in community diversity, though for the 2.8 g/d dose increases in proportions of *F. prausnitzii* and *Akkermansia* at different weeks were detected (Finegold *et al.*, 2014). It is noteworthy that bifidobacteria, total anaerobes, and *Bacteroides fragilis* were higher in the 1.4 and 2.8 g/d treatments, respectively, as estimated by plate culturing. It was suggested that lack of significant effects of XOS on the microbiota by 16S rRNA using the 454 pyrosequencing platform might be due to lack of primer specificity for bifidobacteria (Hooda *et al.*, 2012). It is noteworthy that the total anaerobes cultured would probably be much lower than actually present in this study, and therefore the bifidobacteria increase could be overestimated by culturing. Indeed, in another study, impact of 2 g/d of XOS was evaluated for 8 weeks in healthy and prediabetic subjects using a different sequencing platform and primers, and this also did not show a bifidogenic effect, although it did induce shifts in a number of other genera (Yang *et al.*, 2015) (Table 4).

Recently, the impact of an AXOS-enriched diet on microbiota of overweight and obese subjects with indices of metabolic syndrome using metagenome sequencing indicated increased relative abundance of *Bifidobacterium, Ruminococcus*, and *Lachnospiraceae* compared to baseline after a 4-week intervention (Benítez-Páez *et al.*, 2019). Metagenome analysis showed increases in the presence of bacterial genes involved in vitamin/cofactor production, glycan metabolism, and neurotransmitter biosynthesis after the AXOS intake; together with the additional lipidomics and metabolomics in this study, it appeared that multiple effects of AXOS supported reversing the glucose homeostasis impairment in the subjects. Five other human studies with AXOS utilising FISH or RT-PCR also showed the bifidogenic effect or a trend, and sometimes effects on other groups (Supplementary Table S4). The effect of AXOS varied with respect to other taxa and depended on whether AXOS was supplemented in bread (Walton *et al.*, 2012), or added to drinks (Francois *et al.*, 2012). The inconsistent results might be due to structural differences between AXOS, as it is known from animal studies that the structure of AXOS, i.e. the ratio between arabinose and xylose, impacts its fermentation behaviour (Van Craeyveld *et al.*, 2008). Clearly, further community-wide phylogenetic studies are required for both XOS and AXOS.

In conclusion, in several 16S rRNA-based studies GOS clearly stimulated a strong selective bifidogenic effect and increased relative abundances of few other groups, similar to inulin. Culturing or FISH studies suggest bifidogenic effects also for XOS and AXOS at least. However, there is insufficient data generated yet for XOS and AXOS using sequencing technologies. Thus, it appears too early to draw conclusions on relationships between structures of the latter two fibres and their impact on composition of the human gut microbiota.

Resistant starches, polydextrose, beta-glucans, Arabic gum, konjac glucomannan and PHGG

A large number of studies to date focused on the microbiota modulating effects of glucose-based carbohydrates, namely resistant starches RS2, RS3, RS4 (resistant maltodextrin (RM; or SCF), and polydextrose (PDX), mainly using high-throughput methods (Tables 6 and Supplementary S5).

Five human studies using sequencing investigated RS2 type (high amylose) maize starch which indicated quite some variable changes in abundances of gut bacterial groups, also in comparison to RS4 (Table 6). In order to systematically develop dietary strategies based on RS that modulate the human gut microbiota, the effect of two types of RS, RS2 and RS4, was studied (Martinez *et al.*, 2010). Interestingly, the two different RS types had different effects on the gut community structure; RS4, but not RS2 induced phylum-level changes, significantly increasing proportions of *Actinobacteria* and *Bacteroidetes* while decreasing *Firmicutes* (Martinez *et al.*, 2010; Upadhyaya *et al.*, 2016). On the other hand, RS2 had an opposite effect, showing an increase in the relative abundance of *Firmicutes* while decreasing the relative abundance of *Bacteroidetes* (Martinez *et al.*, 2010). At the species level, RS4 showed increased relative abundance of *B. adolescentis* (in some individuals quite significantly) and *Parabacteroides distasonis*, while RS2 significantly raised the proportions of *R. bromii* and *E. rectale* when compared to RS4. The crossover design revealed that the microbiota responses to RS and their levels varied between individuals, and that the effects were reversible (Martinez *et al.*, 2010). In a study to investigate the potential of RS to reduce intestinal inflammation, RS2 at 8.5 g/d was supplemented to the diet of stunted Malawian children (Ordiz *et al.*, 2015). This increased the relative abundance of *Actinobacteria* and decreased that of *Firmicutes*, however, there was no change in faecal calprotectin suggesting that RS did not reduce gut inflammation in this setting. The impact of 40 g/d of high amylose maize RS2 on adult microbiota, showed mainly increased relative abundance of *Ruminococcaceae* while many other genera decreased; notably a higher relative abundance of the genus *Streptococcus* was associated with an increase in postprandial hormones, and lower relative abundances of *Ruminococcus torques*, *Eubacterium hallii* and *Eubacterium eligens* groups with reduced abdominal adiposity (Zhang *et al.*, 2019).

Another five human studies investigated effects of RS2 from potato starch which showed numerous changes to bacterial groups in the gut microbiota (Table 6). The most in-depth study on RS2 used a combination of -omics approaches, including 16S rRNA gene sequencing, metaproteomics and metabolomics, to gain a broader understanding of microbe-host interplay in response to RS2 supplementation (Maier *et al.*, 2017). Shotgun metagenomics applied to determine the impact of these fibres on the functional capacity of the gut microbiota showed shifts in bacterial gene abundances for genes associated with carbohydrate, amino acid, and lipid metabolism, as well as metabolism of cofactors and vitamins. In a follow up study for this trial, pathway-based metagenomics was performed on a subset of individuals samples to obtain more insight into the functional aspects (Vital *et al.*, 2018). The outcome indicated a framework whereby primary degradation of RS2 was dominated by *Firmicutes*, particularly with *Ruminococcus bromii*, providing SCFAs, notably increased acetate levels which supported the growth of various butyrate producers; H_2-scavenging sulphite reducers and acetogens concurrently increased. Individual responses of gut microbiota were observed in this study to RS2. Other sequencing studies with RS2 in adults were associated with increased relative abundance of *Bifidobacterium* and often *R. bromii,* besides other bacterial group changes (Alfa *et al.*, 2018; Flowers *et al.*, 2019; Venkataraman *et al.*, 2016).

RS2 from either potato (28-34 g/d) or maize (20-24 g/d) were compared in one human study for their effect on faecal butyrate in a 2 week intervention while slowly increasing the dose during the first week (Baxter *et al.*, 2019). The potato RS2 increased relative abundance of some bifidobacterial species, *R. bromii* and *Clostridium chartatabidum* in some individuals, while maize RS2 increased proportions of certain clostridia and *R. bromii*, though it had no effect on bifidobacteria. Those individuals microbiomes that responded with increased relative abundances of *R. bromii* and *C. chartatabidum* for potato RS significantly increased butyrate production on the short term (Baxter *et al.*, 2019).

The effect of a diet enriched with RS2 from both potato and maize with arabinoxylan was studied in adults with metabolic syndrome who consumed a low fibre diet and compared to the low-fibre diet as control (Hald *et al.*, 2016). This RS2-diet significantly increased relative abundance of *Bifidobacterium,* and decreased abundances of several other genera while alpha-diversity decreased. SCFA levels were higher in the stools of the subjects with the RS2-enriched diet while BCFAs (branched chain fatty acids) indicative of protein fermentation were lower.

The study on the impact of RS3 or non-starch polysaccharides (NSPs) on the faecal microbiota of overweight men showed that the RS3 diet was associated with increased relative abundance of *R. bromii* and *E. rectale* in most volunteers as compared to those consuming the NSP diet (Walker *et al.*, 2011). There was marked inter-individual variation as >60% of RS remained unfermented in two volunteers on the RS diet, compared to <4% in the other 12 volunteers; these two individuals also showed low levels of *R. bromii* as assessed by qPCR suggesting involvement of this species in RS3 metabolism. In a follow up study of the same trial, the composition and diversity of the faecal

Table 6. Effect of β-glucan, partially hydrolysed guar gum, resistant starches and polydextrose on human gut microbiota composition.[1]

Type	Dose g/d	Subjects	Trial design and duration	Technology	Outcome (versus control)	Reference
β-glucan (barley)	3 HMW, 3 LMW, 5 LMW	Mildly hyper-cholesterolemic adults, TC 5-8 & LDL-C 2.7-5.0 mmol/l, BMI 20-24 kg/m^2 (n=19)	RCT, CO, placebo-wheat or rice; 5 wk, 4 wk WO	16S rRNA sequencing (Illumina)	No change in alpha-diversity. 3 g HMW: phyla *Bacteroidetes* ↑, *Firmicutes* ↓; *Bacteroides, Prevotella* ↑, *Streptococcus, Dorea* ↓; 5 g LMW, 3 g LMW: no effects	Wang *et al.*, 2016
PHGG	6	Children with ASD (4-9 yr) (n=13)	Non-randomised	16S rRNA sequencing (Illumina MiSeq)	Numerous changes: e.g. *Blautia*, *Acidaminococcus* ↑, *Streptococcus, Odoribacter* and *Eubacterium* (family *Erysipelotrichaceae*) ↓. alpha-diversity ↓	Inoue *et al.*, 2019
RS2 (maize) or RS4 in crackers	33	Healthy adults (n=10)	DB, CO, placebo-native wheat starch; 2 wk run in, 3 wk interventions, with 2 wk WO in between, 2 wk final WO	16S rRNA pyrosequencing (454); PCR-DGGE, qPCR for *Bifidobacterium* spp.	RS2: phyla *Firmicutes* ↑; family *Ruminococcaceae* ↑; *Dorea* ↓. RS4: phyla *Actinobacteria*, *Bacteroidetes* ↑, *Firmicutes* ↓; family *Bifidobacteriaceae*, *Porphyromonadaceae* ↑, *Ruminococcaceae Erysipelotrichaceae* ↓; genus *Bifidobacterium, Parabacteroides* ↑, *Faecalibacterium*, *Dorea* ↓	Martinez *et al.*, 2010
RS2 (high amylose maize)	8.5	Stunted Malawi children (3-5 yr) (n=18)	Non-randomised; comparison with usual diet	16S rRNA sequencing (Illumina HiSeq)	On RS: phyla *Actinobacteria* ↑, *Firmicutes* ↓; families *Coriobacteriaceae* ↑ and *Lachnospiraceae* ↓; genus: *Lactobacillus* ↑; *Roseburia, Blautia*, *Lachnospiraceae* unclassified, *Clostridium* XlVa, *Oscillibacter, Butyricicoccus,* and *Lachnospiraceae incertae sedis* all ↓	Ordiz *et al.*, 2015
RS2 (high amylose maize)	40	Healthy adults (18-55 yr) (n=19)	RCT, DB, CO; placebo starch; 4 wk, 4 wk WO	16S rRNA sequencing (Titanium)	*Ruminococcaceae* ↑; 15 genera ↓ including members of *Anaerostipes, Bacteroides, Blautia, Holdemanella, Coprococcus, Lachnoclostridium, Lachnospiraceae, Erysipelotrichaceae, Paraprevotella, Phascolarctobacterium, Ruminiclostridium* & *Ruminococcaceae*; no change in alpha-diversity	Zhang *et al.*, 2019
RS2 (high-amylose maize, raw potato starch) + AX-enriched diet	20.7	Adults with metabolic syndrome (n=19)	RCT, CO, blinding not possible, control low fibre diet; 4 wk	16S rRNA sequencing (Illumina MiSeq)	*Bifidobacterium* ↑; numerous other genera ↓, e.g. *Bacteroides, Lachnospira, Ruminococcus, Anaerostipes, Butyricimonas*; alpha-diversity ↓	Hald *et al.*, 2016
RS2 (high amylose) in biscuits	20, 25	End-stage renal disease patients (n=20)	RCT, P, control biscuits with regular flour; 20 and 25 g/d in mo 1 and 2, respectively	16S rRNA sequencing (Illumina MiSeq)	*Faecalibacterium* genus ↑	Laffin *et al.*, 2019
RS2 potato (RSP) or maize (RSM)	28-34, 20-24	Healthy young (17-29 yr) adults (n=174)	RCT, P, control accessible corn starch; 2 wk to over few yrs	16S rRNA sequencing (Illumina Miseq)	RPS: *Bifidobacterium faecale/ adolescentis/ stercoris* ↑, *R. bromii, Clostridium* type ↑ in subset; RSM: *R. bromii* ↑	Baxter *et al.*, 2019

Table 6. Continued.

Type	Dose g/d	Subjects	Trial design and duration	Technology	Outcome (versus control)	Reference
RS2 (*Solanum tuberosum* extract)	30	Elderly (n=20) and mid-aged adults (n=20)	RCT, DB, P; placebo corn starch; 3 mo	16S rRNA sequencing (Illumina MiSeq)	*Bifidobacterium* ↑; in elderly only *Proteobacteria* (*Escherichia coli/Shigella*) ↓, *Prevotella* ↑, *Alistipes* ↑, *Desulfovibrio* ↑, *Mogibacterium* ↑, *Sporobacter* ↑; in mid aged only *Olsenella* ↑, *Coprobacillus* ↓, *Lactobacilllus* ↓	Alfa *et al.*, 2018
RS2 (*Solanum tuberosum* tuber)	HRS: 66 or 4; LRS: 48 or 3	Insulin resistant adults on low (n=23) or high (n=16) carbohydrate diets; n=12 for meta-genomics of LC arm	RCT, CO, time series study with LRS versus HRS; 2 wk run-in, 2 wk intervention, 2 wk WO	16S rRNA sequencing (Illumina HiSeq 2000); metagenomics	*Firmicutes* ↑, *Bacteroidetes* ↓ (*Faecalibacterium prausnitzii, Prevotellaceae, Ruminococcus, Eubacterium rectale, Roseburia faecis, Akkermansia muciniphila* ↑); metagenomics: see text	Maier *et al.*, 2017; Vital *et al.*, 2018
RS2 (unmodified potato starch)	12, 24, 48 (50% RS2)	Healthy young adults (n=20)	Non-randomised; control-habitual diet; gradual increase for 3 d, then 7 d total dose	16S rRNA sequencing (Illumina MiSeq)	Individuals classified into enhanced, high, & low (n=11, n=3, and n=6, respectively) based on butyrate levels before and during RS; *B. adolescentis* or *R. bromii* ↑ in enhanced & high; in 5 subjects, also *E. rectale* ↑; no diversity change	Venkataraman *et al.*, 2016
RS2 (raw unmodified potato starch)		AAP-treated bipolar disorder/ schizophrenic adult patients (n=37)	Cross-sectional cohort study, control-habitual diet; 14 d	16S rRNA sequencing (Illuminia MiSeq platform)	*Actinobacteria* ↑, *Bacteroides* & *Parabacteroides* OTUs ↓, ↑ in RS degraders *Bifidobacterium faecale*, *B. adolescentis*; some individuals *R. bromii* & OTU *Clostridium* cluster IV ↑; alpha-diversity no change	Flowers *et al.*, 2019
RS3 versus wheat bran (NSP) in food	RS 50-60 or NSP 40-54	Overweight men (n=14); 10 w on different diets	RCT, CO; 7 wk maintenance diet, 3 wk intervention, then 3 wk weight loss diet	16S rRNA sequencing (Illumina MiSeq; qPCR for various genera/ groups including bifidobacteria, clostridial clusters (XIV, IV), *Roseburia* spp., *E. hallii*, 4 bifidobacterial species, total bacteria; HITChip microarray	Sequencing: *Ruminococcus, Roseburia* ↑ on RS diet. no effect on *Bifidobacterium* of RS; no effects of NSP. HITChip: RS: *Ruminococcaceae* ↑; NSP: *Lachnospiraceae* ↑; RS: diversity ↓	Walker *et al.*, 2011; Salonen *et al.*, 2014
(RS4) RM in drink	25, 50	Healthy males (n= 14)	RCT, CO, DB; 3 treatments: 50 g/d maltodextrin control, 25 RM+25 g/d maltodextrin, 50 RM+0 g/d maltodextrin; 24 d intervention, ≥2 wk WO between	16S rRNA pyrosequencing (454 titanium); FISH for total bacteria and major groups of *Bacteroidetes, Clostridiales,* bifidobacteria; qPCR for bifidobacteria; PCR-DGGE	Sequencing: *Ruminococcus, Eubacterium, Lachnospiraceae*, *Bacteroides, Holdemania, Faecalibacterium* ↑. FISH, qPCR: no effect on *Bifidobacterium* (some bifidobacteria ↑ from baseline for all); PCR-DGGE-band sequencing: OTU related to *Lachnospiraceae*	Baer *et al.*, 2014; Culpepper *et al.*, 2012
RS4 in flour	30% v/v	Adults with metabolic syndrome signs (n=20)	RCT, CO, DB, placebo-control flour; 2×12 wk, 2 wk WO	16S rRNA sequencing (MiSeq)	Differential abundance of 71 OTUs, including ↑ 3 *Bacteroides* species, one each of *Parabacteroides, Oscillospira, Blautia, Ruminococcus, Eubacterium* & *Christensenella* species	Upadhyaya *et al.*, 2016

Table 6. Continued.

Type	Dose g/d	Subjects	Trial design and duration	Technology	Outcome (versus control)	Reference
SCF (RS4) in muffin and fruit drink	10 or 20	Female adolescents (11-14 y, n=30)	3-phase, RCT, DB, CO; placebo-maltodextrin in beverages, muffins prepared per the recipe with no placebo; 4 wk each treatment, 3 to 4 wk WO periods	16S rRNA sequencing (Illumina MiSeq)	*Bifidobacterium* ↑ with 20 g/d; *Parabacteroides, Anaerostipes, Ruminococcus, Lachnospiraceae* ↑ with 10 and 20 g/d; *Dorea, Dialister* ↓ with 20 g/d; diversity ↑ with both dosages	Whisner *et al.*, 2016
SCF (RS4); or as synbiotic with *Lactobacillus rhamnosus* GG or *L. rhamnosus* GG-PB12	6	Healthy elderly (n=37)	RCT, DB, CO, placebo-maltodextrin; 2 wk run-in, 3 wk intervention, 3 wk WO	16S rRNA sequencing (Illumina MiSeq)	SCF: *Ruminococcaceae incertae sedis* ↑; synbiotic: *Parabacteroides* ↑, *Ruminococcaceae incertae sedis* ↑, *Oscillospira* ↓, *Desulfovibrio* ↓	Costabile *et al.*, 2017
PDX or SCF (RS4) in snack bar	3x7 in snack bars	Healthy males (n=20)	RCT, DB, CO, placebo-bar with no supplemental fibre; 21 d, no WO	16S rRNA pyrosequencing (454 Titanium)	*Clostridiaceae*, *Veillonellaceae*, ↑; *Faecalibacterium (prausnitzii)*, *Phascolarctobacterium*, *Dialister* ↑; *Eubacteriaceae* ↓; *Bifidobacterium* ↓; SCF only: *Lactobacillus* ↑	Hooda *et al.*, 2012
PDX or SCF (RS4)	21	Healthy adult men (n=21)	RCT, DB, 3-period CO, placebo-bar with no PDX/SCF; 21 d	Whole-genome shotgun pyrosequencing (454)	*Bacteroidetes* ↑	Holscher *et al.*, 2015b
PDX or PDX + probiotic	12	Obese and overweight adults (n=72)	RCT, DB, P, placebo-cellulose; 6 mo	16S rRNA sequencing (Illumina Miseq)	PDX: ↑ *Christensenellaceae* at 2,4, 6 mo, *Methanobrevibacter, Parabacteroides, Rikenellaceae*, uncultured *Ruminococcaceae* at 2 time points. PDX+probiotic: *Akkermansia* ↑, *Christensenellaceae* ↑, *Methanobrevibacter* ↑; *Paraprevotella* ↓	Hibberd *et al.*, 2019

[1] AAP = atypical antipsychotic treatment; AX = arabinoxylan; CO = crossover; DB = double blind; FISH = fluorescent *in situ* hybridisation; HC/LC = high/low carbohydrate; HMW = high molecular weight; LDL-C = LDL-cholesterol; LMW = low molecular weight; NSP = non-starch polysaccharides; OTU = operational taxonomic unit; P = parallel; PCR-DGGE = PCR-denaturing gradient gel electrophoresis; PDX = polydextrose; PHGG = partially hydrolysed guar gum; qPCR = quantitative PCR; RCT = randomised controlled trial; RS2 = type 2 resistant starch, native granular starch; RS3 = type 3 resistant starch, retrograded starch; RS4 = type 4 resistant starch, chemically modified starch; RM = resistant maltodextrin; RSM = resistant starch maize; RSP = resistant starch potato; SB = single-blind; SCF = soluble corn fibre; TC = total cholesterol; WO = washout; ↑↓ = significantly increased or decreased.

microbiota were analysed by the phylogenetic HITChip microarray and qPCR which again showed marked changes (Salonen *et al.*, 2014). The RS increased relative abundances of multiple phylotypes of *Ruminococcaceae*, whereas that of most *Lachnospiraceae* phylotypes were increased in the NSP group. In addition, the RS3 diet decreased the diversity of the microbiota significantly and the dietary responsiveness of the individual's microbiota was inversely associated with its microbiota diversity. Both of these studies noted that RS3 had a marked effect on the microbiota, and that the effect was influenced by the initial microbiota composition of each individual, possibly predisposing them to be a responder or a non-responder (Salonen *et al.*, 2014).

There were five human 16S sequencing studies for RS4 (Table 6). The impact of 25 or 50 g/d RM (RS4) compared to maltodextrin on faecal microbiota was evaluated using a combination of 16S rRNA, FISH, and qPCR (Baer *et al.*, 2014). Sequencing showed significant increases in proportions of various operational taxonomic units (OTUs) matching closest to *Ruminococcus, Eubacterium, Lachnospiraceae, Bacteroides, Holdemania* and *Faecalibacterium,* suggesting a broad impact of RM on the gut microbiota. FISH showed that there was a dose-dependent increase in total counts of faecal bacteria, and faecal wet and dry weight also increased significantly. In another study, RS4 supplementation was associated with enrichment in relative abundance of numerous species, including *P. distasonis*, *Christensenella minuta*, *Bacteroides ovatus, Bacteroides xylanisolvens*, *Bacteroides acidifaciens, Ruminococcus lactaris, B. adolescentis*, *Eubacterium oxidoreducens* and other OTUs within genera *Ruminococcus*, *Blautia, Bacteroides, Oscillospira,* and *Parabacteroides* (Upadhyaya *et al.*, 2016). This study also showed increased relative abundance of *Clostridial* cluster XIVa, a group which includes taxa associated with the gut mucosal layer (Upadhyaya *et al.*, 2016). Interestingly the RS4 intervention had a significant effect on adipocytokines which play a role in lipid and glucose metabolism and help determine progression to cardiovascular aberrancies (Upadhyaya *et al.*, 2016). In a study with 10 or 20 g/d soluble corn fibre (SCF) in pubertal females, significant increases in *Bifidobacterium* from mean proportion of 3.2 to 5.10% and *Dialister* (from 0.5 to 1.2%) were found with 20 g/d, whereas *Parabacteroides* and *Lachnospiraceae* proportions increased with both dosages, while *Dorea, Anaerostipes* and *Ruminococcus* proportions decreased after consumption of the SCF (Whisner *et al.*, 2016). Significant changes in global microbiota were also reported in elderly subjects upon intervention with 6 g/d SCF alone, and in combination with two probiotic strains of *Lactobacillus rhamnosus* (Costabile *et al.*, 2017).

Three sequencing studies investigated PDX. Impact of both PDX and SCF (RS4) fibres on the composition of faecal microbiota of 20 healthy adult men with a mean dietary fibre intake of 14 g/d for 21 d was studied using 454 sequencing (Hooda *et al.*, 2012). The study showed that the consumption of both fibres led to higher relative abundance of faecal *Clostridiaceae* and *Veillonellaceae*, and lower relative abundance of *Eubacteriaceae*, as compared to the no fibre control. The relative abundance of *Faecalibacterium, Phascolarctobacterium* and *Dialister* was greater in response to polydextrose and SCF intake. Among *Actinobacteria*, relative abundances of the families of *Bifidobacteriaceae* and *Coriobacteriaceae* were decreased for both fibres as compared to the control, and the relative abundance of *Clostridium* and *Akkermansia* were increased upon PDX consumption, while higher relative abundance of *Lactobacillus* was observed only in the SCF group. Thus, there were marked effects on the faecal microbiota at the class, genus, and species level due to both fibres. In a follow up study on the same intervention for PDX and SCF, the faecal samples were subjected to whole-genome shotgun pyrosequencing to identify both faecal bacterial populations present and their functional genetic capacity (Holscher *et al.*, 2015b). Both fibres shifted the *Bacteroidetes*:*Firmicutes* ratio, significantly increasing the relative abundance of *Bacteroidetes* (and in particular *Porphyromonadaceae*) 12 ± 2% (polydextrose) and 13 ± 2% (SCF) as compared to the no fibre control. The effects of PDX were also evaluated using FISH, qPCR and DGGE techniques which in fact gave somewhat different outcomes (Costabile *et al.*, 2012). The DGGE data showed that there were significant differences in the indices of the placebo versus the PDX treatment; qPCR analyses showed a significant increase in the butyrate producer *Ruminococcus intestinalis*, *Clostridium histolyticum* (clusters I and II) group and *Clostridium leptum* (cluster IV) for PDX in comparison to the placebo; the FISH analyses did not show significant differences for PDX versus placebo. The most recent human study using sequencing compared 12 g/d of PDX with and without a probiotic to cellulose in overweight or obese individuals (Hibberd *et al.*, 2019). The most notable outcome was increased prominent abundance of *Christensenellaceae* for PDX at the 2, 4 and 6 month end of intervention, also with the probiotic combination, and the latter correlated negatively to waist-area body fat mass after 6 months treatment with LU+B420.

The impact of high and low molecular weight barley beta-glucans on the faecal microbiota of mildly hypercholesterolemia subjects was investigated to determine if there was an association with improving risk factors in cardiovascular disease (Wang *et al.*, 2016). Only the high molecular weight beta-glucans at 3 g/d induced shifts, namely increased relative abundance of *Bacteroides* and decreased *Dorea* with a reducing trend in *Prevotella*. This altered profile was associated with a reduction of cardiovascular risk markers, such as BMI, total cholesterol and blood pressure amongst others. Recently, PHGG supplementation in children with autistic spectrum disorder showed numerous changes, such as increased relative abundance of *Blautia* and *Acidaminococcus*, and decreases in that of *Streptococcus, Odoribacter* and *Eubacterium* (family *Erysipelotrichaceae*) (Inoue *et al.*, 2019). There was significant improvement in 'behaviour irritability' and constipation symptoms in the children. Finally, there were no high-throughput studies evaluating the microbial effects of Arabic gum and konjac glucomannan. However, qPCR based studies showed that consumption of Arabic gum and konjac glucomannan could increase levels of bifidobacteria (Table S5) (Calame *et al.*, 2008; Wu *et al.*, 2011).

5. Concluding discussion

The human gut microbiota is emerging as an important factor contributing to human physiology. Microbiota modulation by foods and dietary supplements may provide an attractive way to support health. Dietary fibres and prebiotics by their nature are food for the human colonic microbiota and thereby may play a key role. The establishment of increasingly advanced and cost-effective molecular technologies provides an opportunity to explore the impact of fibres on the human colonic microbiota. Interestingly, based on this review of the 16S rRNA-based studies on microbial effects of prebiotics and specific fibres, the inulin-type fructans and GOS, each composed of different monosaccharides, have strong selective bifidogenic effects with apparent lesser effects on the gut microbial community as a whole. Thus, there appears to be no clear relationship between the chemical structure and composition of at least some fibres and their effect on the microbiota, as has been previously noted (Hamaker and Tuncil, 2014). In contrast, numerous broad sequencing technologies show that the glucose-based fibres, such as different RS types and PDX, have broader effects and more diverse effects on the gut microbiota, with notably more stimulation of *Ruminococcus* spp., specifically *R. bromii* on RS2, as compared to inulin and GOS. This outcome is in line with the outcome of a recent systematic review whereby the subgroup analyses of common prebiotics like inulin and GOS versus other dietary fibres, showed that the common prebiotics led to significantly greater relative abundance of *Bifidobacterium* and *Lactobacillus* spp. (So *et al.*, 2018). There are currently insufficient studies on gut microbiota performed for some fibres/NDOs, such as β-glucans, PHGG, AXOS and XOS and the outcomes reported thus far are not consistent enough to conclude their effects.

The effect of prebiotics, for example inulin-type fructans on specific microbial groups, such as *Bifidobacterium* spp., *Lactobacillus* spp., and common pathogenic taxa has been investigated for decades, but the community-wide shifts are only recently investigated. *Bifidobacterium* spp. is a genus that has coexisted with the mammalian intestine over thousands of years indicating a strong symbiosis between this microbe and the human body (Moeller *et al.*, 2016). Bifidobacteria show broad capacity for carbohydrate breakdown and are well adapted to a glycan-rich environment in the gut (El Kaoutari *et al.*, 2013; Milani *et al.*, 2016). Inulin-type fructans, recognised as prebiotics, were shown to be associated with bifidogenic effects a long time ago (Gibson and Roberfroid, 1995; Hidaka *et al.*, 1986). Such selective effects are confirmed by the newer 16S rRNA-based high-throughput sequencing methods, as shown in this review. Initially inulins were valued for improving digestive health or bowel habit, that has been more recently substantiated by a systematic review (De Vries *et al.*, 2019). Importantly there is an increasing evidence for inulins' role in glucose homeostasis relevant against type 2 diabetes and obesity, amongst several other benefits (Canfora *et al.*, 2015; Koh *et al.*, 2016; Liu *et al.*, 2017b; Slavin, 2013; Stephen *et al.*, 2017; Van de Wouw *et al.*, 2018; Van der Beek *et al.*, 2018). Microbes do not act in isolation and via the process of cross-feeding and other beneficial and/or antagonistic interactions they can influence other members within the community (Tims *et al.*, 2016). This can explain how increases in levels of non-butyrate producing *Bifidobacterium* can stimulate activity of butyrate producing taxa and thus correlate with higher levels of butyrate production upon fibre intervention providing health benefiting effect to the host (Alfa *et al.*, 2018; Tims *et al.*, 2016). Thus, in order to gain a more comprehensive view on how dietary fibres influence the gut community structure and function, the application of high-throughput and -omics methods became necessary.

The dietary fibre/NDO imposed changes in microbiota composition will especially affect the saccharolytic fermentation and SCFA production, and these effects will benefit the metabolic and physiological status of the host, for example by improving bowel habit or insulin resistance, amongst others (Canfora *et al.*, 2015; Koh *et al.*, 2016). However, the reported changes in microbiota composition in relation to dietary fibre/ NDO intake can be inconsistent between studies and subtle, and individual-specific, as shown in this and other reviews (Makki *et al.*, 2018). The effect of different fibres on microbial diversity clearly varies. For example, the microbial alpha-diversity increased in interventions with SCF (RS4) (Whisner *et al.*, 2016), while the microbial diversity declined when chicory inulin (Reimer *et al.*, 2017; Vandeputte *et al.*, 2017), GOS (Liu *et al.*, 2017a) or RS3 (Salonen *et al.*, 2014) were used. There was no effect on diversity in other human studies for chicory FOS (Tims *et al.*, 2016), GOS (Canfora *et al.*, 2017; Grimaldi *et al.*, 2018; Krumbeck *et al.*, 2018; Liu *et al.*, 2017a), RS2 (Morales *et al.*, 2016; Venkataraman *et al.*, 2016) or RS4 (Upadhyaya *et al.*, 2016). The differences also include the magnitude of changes in abundance of individual taxa (notably *Bifidobacterium*) and individual differences in responses between study subjects, such as was observed for inulin and (Fuller *et al.*, 2007; Healey *et al.*, 2018; Kolida *et al.*, 2007) GOS in several studies (Azcarate-Peril *et al.*, 2017; Davis *et al.*, 2011; Liu *et al.*, 2017a; Pedersen *et al.*, 2016), and FOS (Liu *et al.*, 2017a), and RS3 (Salonen *et al.*, 2014). Subjects' basal diets may also affect responsiveness to inulin-type fructan supplementation as it was observed that the bifidogenic effect seems to be greater in subjects with a high habitually dietary fibre intake (Healey *et al.*, 2018) and those with lower initial bifidobacteria levels (De Preter *et al.*, 2008; Kolida *et al.*, 2007; Tuohy *et al.*, 2001a,b). It was also observed that GOS with higher purity (from 60 to >95% of fibre) gave a bifidogenic effect and occasionally other changes, while with GOS of low purity (32%) there was no bifidogenic effect (Table 5). Thus, the different outcomes between human studies with similar fibres/NDOs, and between responders and non-responders in the studies, may be related to numerous factors, including initial presence/abundance of keystone microbial species in the community (De Preter *et al.*, 2008; Korpela *et al.*, 2014b; Meyer and Stasse-Wolthuis, 2009; Roberfroid *et al.*, 2010; Salonen *et al.*, 2014), diet (Healey *et al.*, 2018; Salonen *et al.*, 2014), gender (Upadhyaya *et al.*, 2016), intervention length, health status of the subjects, (sufficient) dose of fibres used (Davis *et al.*, 2011; Depeint *et al.*, 2008; Makki *et al.*, 2018) or the form that the fibre is delivered during the study (Francois *et al.*, 2012; Walton *et al.*, 2012). Furthermore aspects of the technologies may also play a role in the discrepancy between outcomes for sequencing studies, for example certain primer pairs may be insufficient for bifidobacteria, and the disruption of bifidobacterial cells to release DNA/RNA may also affect the detection and levels (Chen *et al.*, 2019; Walker *et al.*, 2015).

Consequently, to understand and address the inconsistencies in research outcomes, a development of systematic approaches to fibre and microbiome studies is needed (Klurfeld *et al.*, 2018). This should include subjects' microbiota screening and stratification at enrolment (Reid *et al.*, 2010), as well as precise characterisation and monitoring of baseline diets used by the study participants, and regular longitudinal microbiota sampling during the intervention. The specific responses to different fibres, as well as proper fibre dosing need to be further investigated. Details of sequencing platforms should be provided. This knowledge could be then applied to support development of systematic approaches in screening of novel dietary fibres and could provide bases for more accurate and relevant definition of prebiotic fibres in the future.

The concept of gut health is complex and it combines diet, microbiota and host mucosa (Valdes *et al.*, 2018). Thus, studies on food ingredients and their effects on microbiota and host require the use of multidisciplinary approaches. The field of microbiota research is rapidly growing due to increasing availability and affordability of novel diagnostics and rising interest from government, industry and public sector. An increasing wealth of technologies with integrated 'omics' approaches are available to study effects of food ingredients on the gut microbiome. Metabolomics and proteomics are capable of detecting and tracking diverse microbial metabolites from different non-digestible food ingredients, discriminating between phenotypes with different inherent microbiota, and potentially diagnosing gastrointestinal state (Jacobs *et al.*, 2009). Metagenomics and metatranscriptomics can characterise the genetic potential and activity of numerous gut microbial species. Systems immunology can be applied to characterise the physiological effect of microbial substrates on markers involved in the inflammatory, autoimmune, allergic and other immune-related conditions in the host (Davis *et al.*, 2017), and to indicate beneficial health outcomes. Thus, integration of several omics techniques is a further step towards a more coherent understanding of the complex microbe-host mutualism. Together all these approaches could lead to identification of a set of biomarkers that could adequately indicate functional presence of beneficial microbes, or their metabolites, and their host effect in relation to specific dietary components (Celi *et al.*, 2018).

In conclusion, the adaptation of high-throughput 16S rRNA-based technologies allows superior monitoring of changes in the overall microbial community diversity due to fibre consumption. High microbial diversity contributes to ecosystem stability, resilience and host health. The structural and chemical complexity of carbohydrates in the gut is likely to provide competitive advantage to other taxa adding to the complexity of the fibre mediated responses in the gut community (Hamaker and Tuncil, 2014). The widespread use of Western diets in modern societies has been associated with loss of microbial diversity and large-scale imbalances in the gut microbiota (dysbiosis) (Sonnenburg *et al.*, 2016). Fibres carry a promise to prevent or reverse the diversity loss, and through their microbial effects might even offer therapeutic potential with a wide range of applications, for example in offsetting the negative impacts of antibiotic therapies, facilitating effectiveness of faecal microbiota transplants treatments or alleviating symptoms of inflammatory bowel disease (Wong *et al.*, 2016). Although this review focused on defined added fibres, studies with diets enriched with mixes of dietary fibres are providing valuable information on fibre impact on health. When African Americans were switched from their usual low-fibre/high-fat diet to a high-fibre/low-fat diet, changes in mucosal biomarkers of cancer risk and in aspects of the microbiota and metabolome known to affect cancer risk were observed; there was increased saccharolytic fermentation and production of butyrate and suppression of secondary bile acid synthesis (O'Keefe *et al.*, 2015). Adopting a high-fibre diet in diabetic humans promoted changes in the entire gut microbe community, stimulated the growth of SCFA-producing organisms, and this correlated with elevated levels of glucagon-like peptide-1, a decline in acetylated haemoglobin levels, and improved blood-glucose regulation (Zhao *et al.*, 2018). More recently, interventions with whole-grain or white breads were performed which showed that the type of bread that induces the lower glycaemic response in each person could be predicted based solely on microbiome data prior to the intervention. This marked personalisation in both bread digestion and the gut microbiome, strongly suggests that understanding dietary effects requires integration of person-specific factors (Korem *et al.*, 2017). Clearly, there is tremendous promise for prebiotics and fibres directed at the gut microbiome for general nutrition to personalised beneficial effects to improve or maintain human health.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2019.0082.

Table S1. Gut microbiota analyses technologies reviewed by Fraher *et al.* (2012) and Sekirov *et al.* (2010).

Table S2. String searches in PubMed (10 Sept 2019) for single/ added fibres and prebiotics.

Table S3. Effect of inulin-type fructans on human gut microbiota composition.

Table S4. Effect of AXOS, GOS and XOS on human gut microbiota composition.

Table S5. Effect of Arabic gum, konjac glucomannan, PDX, PHGG, and RS4 on human gut microbiota composition.

Conflict of interest

This paper was based on an expert panel discussion held in Chicago in 2016 which was financially supported by Sensus B.V. and a subsistence fee was provided to the following authors: ECM, WMdV, KSS, JG, PDM, JH, and JLS. These authors have no conflict of interest to declare. KB supported updating of the data and writing. EEV is an employee and PDM was a former employee of Sensus. The opinions expressed herein, and the conclusions of this publication are those of the authors.

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