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PREBIOTICS: MODULATORS OF THE GUT MICROBIOME

The intentional modulation of the gut microbiome for targeted health outcomes is now considered to be a viable therapeutic strategy for a wide-range of dysfunctions; for many gastrointestinal conditions, as well as for nearly every other organ system in the body. These therapies are intended to leverage the vast numbers of metabolites and signaling molecules that are generated by the gut microbiota known to influence important physiological processes within the host. In general, when the gut microbial ecosystem is robust, balanced and diverse, it generates signals that promote healthy outcomes in the host; while a weak, poorly balanced ecosystem (i.e.: dysbiosis) promotes many unhealthy signals and is associated with the prevalence of many chronic diseases. In general, there are three ways to modulate the human gut microbiota to increase diversity and expand metabolic benefits to the host; remove harmful agents that promote dysbiosis (e.g., certain drugs, antibiotics, pathogens, etc.), add live microorganisms that can promote a healthy ecosystem (i.e., probiotics) or provide substrates which can be utilized by commensal organisms to promote a healthy microbial ecosystem. This last therapeutic category is generally known as "Prebiotics", of which the definition, mechanisms of action and clinical potential are the focus of this monograph.

Prebiotics: Definitions Abound

As with many terms used by researchers, reporters, regulators, healthcare professionals, consumers and marketers alike, the definition of a "prebiotic" is quite varied, confusing and, in some cases, contentious.[1] Numerous organizations have been working to define a consensus definition for the term prebiotic, with the hopes of gaining wide acceptance amongst key stakeholders.[2] First, we will describe the "strict" definition of a prebiotic, which is gaining traction amongst global organization, regulators and researchers, before identifying other microbiome-modulating ingredients that may not meet these strict criteria (though they are often marketed as prebiotics).

The term "prebiotic" is relatively new, being first defined in 1995 by Gibson and Roberfroid as a "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health."[3] While there were earlier description of this activity (often referred to simply as "bifidogenic"), this definition attempted to define this new term vis-à-vis the already adopted definition of a probiotic, and further introduced the term "synbiotic" as a combination of these two (i.e., delivery together of both pre and probiotics).[4,5] The last 30 years have seen numerous challenges, disputes, refinements and a host of new proposed definitions for the term. These have been posited mostly in the attempt to include alterations of microbiota in other sites beyond the colon (small bowel, oral, vaginal, etc.), to broaden (or remove) the term "selectively," and nuance ways to define what specific changes in the microbiota are deemed beneficial to the host.

Not surprisingly, questions as to how to further refine parts of this definition, such as a "nondigestible food ingredient" or "selectively stimulating…a limit number of bacterial species" began to be debated.[6] At the same time, research discovering other nutrient-related influences to host gastrointestinal (GI) microbiota from substances that did not meet the criteria of "non-digestible" food ingredients began to be published- several of which were deemed "prebiotics." The International Scientific Association for Probiotics and Prebiotics (ISAPP) has adopted a slight variation of the original definition which is "a substrate that is selectively utilized by host microorganisms conferring a health benefit."[7] According to ISAPP, this definition expands the concept of prebiotics to possibly include non-carbohydrate substances (e.g., polyphenols), applications to body sites other than the gastrointestinal tract, and diverse categories other

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than food; but retains the requirement for selective microbiota-mediated mechanisms. However, the International Probiotics Association (IPA) has advocated for a more restricted definition that requires a prebiotic meet five criteria: 1) resistant to digestion by the host, 2) not absorbed in the GI tract by the host, 3) is fermentable or metabolized by certain gut microbes, 4) has a direct, beneficial and selective effect on beneficial microbes, and 5) has a beneficial physiological effect on the host when consumed in adequate amounts.[2,†]

Finally, the Global Prebiotic Association (GPA)- a group representing manufacturers of prebiotic ingredients- has proposed a definition which is perhaps closest to how the term is used by clinicians and consumers, which is "a compound or ingredient that is utilized by the microbiota producing a health or performance benefit."[1] In addition, they say that a prebiotic should have "a health or performance benefit that arises from alteration of the composition and/or activity of the microbiota, as a direct or indirect result of the utilization of a specific and well-defined compound or ingredient by microorganisms." This definition implies that a prebiotic must demonstrate some microbiome-modulating effect that can then be connected, mechanistically, to a measurable benefit for the host consuming the prebiotic.

While these definitions are important for ensuring that those discussing prebiotics are communicating clearly and speaking about the same category of therapeutic ingredients, we will now turn our attention to describing compounds that meet one or more of these definitions of prebiotic. Note that nearly all of these compounds are plant-derived, which is why higher microbiota diversity is nearly always associated with diets higher in plant consumption (both volume and variety).[8]

Dietary and Fermentable Fibers

For many years, a prebiotic was commonly assumed to be nearly synonymous with "dietary fiber." And while most prebiotics can be categorized within the family of fiber compounds, the definitions discussed above radically alters the list of fibers that meet these various definitions (from as few as two to as many as a dozen or more)[9,10,11] By the strict definitions mentioned above, a fiber ingredient must be fermentable to be considered a prebiotic. However, many outcomes related to consuming fiber can indirectly influence the gastrointestinal environment in ways that affect the microbiota without direct fermentability. Two important ways would be altering bowel transit time and their ability to modulate the absorption of glucose (or other dietary sugars) such that they are less available for fermentation.

As a category, fiber represents a diverse range of compounds (e.g., celluloses, gums, glucans, pectins, etc.) with varying physiochemical properties (e.g., solubility, viscosity when hydrated, and fermentability); and are derived from a diverse range of sources, mostly plants, but animals (e.g., chitin) and even synthetic sources may also qualify.[12] Because of this diversity, there has been controversy amongst nutritional scientists and regulatory agencies as to the technical definition of dietary fiber. In 2001, the IOM distinguished "dietary fiber" as "*nondigestible carbohydrates and lignin that are intrinsic and intact in plants*" (e.g., lignin, cellulose, beta-glucan, etc.) from "functional fiber" defined as, "*isolated, nondigestible carbohydrates that have beneficial physiological effects in humans*" (e.g., psyllium, chitin, fructooligosaccharides, etc.).[13] The summation of "dietary fibers" and "functional fibers" gives rise to the IOM's total fiber definition. Again, these categories are controversial, and some prefer to classify fiber into specific categories based on physiochemical properties: (1) insoluble, poorly fermented; (2) soluble, non-viscous, readily fermented; (3) soluble viscous/gel forming, readily fermented and (4) soluble viscous/gel forming, nonfermented. These categories give rise to differences in the biological activities of fiber such as its effect on blood glucose homeostasis, stool bulking, laxative effects and the ability of fiber to bind bile acids. Differing analytical methods have also been a source of controversy in classifying dietary fiber.

Regardless of the controversy surrounding the definition of fiber, the IOM established Adequate Intake (AI) levels for dietary fiber based on large cohort studies that found significant reductions in the risk of coronary heart disease with fiber intakes of approximately 14 g/day per 1,000 kcal consumed.[14-17] No tolerable upper intake level has been set by the IOM for fiber. Despite these recommended intakes, many clinicians and nutritionists believe the IOM's Adequate Intakes for fiber are much too low to maintain optimal health, recommending instead 45 or even 50 grams of fiber per day. However, even compared to current AI levels, most Americans are not reaching the IOM's benchmark levels of total fiber. Specifically, according to NHANES data from 2015 – 2016, reported fiber intakes for adults ≥ 20 years was 8.0 g fiber per 1,000 calories for males and 9.0 g fiber per 1,000 calories for females.[16] Further, a report from the Institute of Medicine, notes only 3% of Americans consume adequate amounts of fiber and that average fiber consumption is only half the recommended intake amount (i.e., only 7 grams/1,000 calories), similar to what is shown in the NHANES data above.[17]

Fiber intake amounts may be much lower for individuals consuming a low-carbohydrate diet, as some estimate fiber intakes in this group are as low as 10 g/day.[12] Indeed, fiber has been classified as an under- consumed nutrient of public health concern since the 2010 – 2015 Dietary Guidelines for Americans.[18] This preponderance of inadequate fiber intake is concerning due to the numerous health benefits of fiber consumption. In both men and women, the risk of metabolic

† For scientific clarity, IPA has recently adopted ISAPP's 2017 definition for a prebiotic

syndrome, inflammation and obesity is significantly lower for those consuming fiber in the highest quintile of intake (> 22.5 grams) compared to the lowest quintile of intake (< 8.1 grams).[19]

It is important to note that the biological activities of fiber (e.g., benefits on blood glucose levels, lowering serum cholesterol, modifying the gut microbiota composition, laxative effects, etc.) are dependent upon their specific physiochemical properties.[20] Beyond their water holding capacity, soluble fibers influence physiological functions such as glucose homeostasis, stool viscosity, and their ability to be fermented by various gut microbes (i.e., their "prebiotic" effect).[21] Further fiber can positively influence the binding capacity of bile and anion exchange capacity. Since most of the data suggesting fiber intake is associated with reduced incidence of chronic health conditions draws from epidemiology, it is difficult to know whether it is specifically the fiber content of foods or other properties of fiber-rich foods (e.g., phytonutrients) that contributes to every beneficial health effects – for this reason increased consumption of fiber rich foods is considered as the first-line recommendation for increasing fiber intakes, followed by supplemental fiber intakes.

The role of dietary fiber from food is vital for maintaining balanced blood sugar levels, especially in type 1 and type 2 diabetic subjects. Large prospective cohort studies consistently show associations of a high dietary fiber intake (>25 g/d in women and >38 g/d in men) with a 20-30% reduced risk of developing type 2 diabetes.[22] Mechanistically, fiber slows glucose absorption in the small intestine and reduces postprandial blood glucose levels. Decreased fiber intake due to modern food practices is suggested to be the leading cause of idiopathic constipation. While this has been empirically shown in adults for more than a century, this has been researched specifically in children. In one study in Brazil, children with chronic constipation typically ingested 20 – 30% less fiber than age- and gender-matched controls with normal bowel habits.[23] In a larger sample group of children in Greece, dietary fiber alone was independently and inversely correlated with chronic constipation, despite the child's age or age of onset of constipation.[24] Furthermore, a study conducted in the U.S. showed constipated children were consuming less than 25% of the age-plus-five recommended fiber intake, even though they had been instructed "to eat a high-fiber diet." It seems in children, as well as adults, increasing dietary fiber intake is a difficult lifestyle change that is not often achieved through dietary changes. Research in children and adults show that constipation is associated with changes of the species abundance or to the metabolites (i.e., short-chain fatty acids) produced by the gut microbiome.[25,26]

The most common supplemental forms of fiber are psyllium (seed and husks), flaxseeds, beta-glucans (oats or other grains), inulin and oligofructose, fruit pectin concentrates, chitosan (shellfish), gums (e.g., guar gum) and a variety of celluloses. Many of these are ingredients commonly found in products sold as prebiotic fibers. Combining soluble, insoluble and fermentable fibers (all contribute to the dietary fiber total), is the best way to promote the wide-ranging benefits of increased fiber intake. Clinicians should have specific recommendations or formulary options available which contain several fiber-containing supplements. Products sold as "powders" to be mixed in water or juice will be easier to consume in higher doses, although some patients prefer encapsulated or tableted products. Note that it often takes 12 or more capsules or tablets to reach the same dose as a single scoop of many commercially available fiber powders. Clinicians should be aware that high levels of fermentable fibers are not tolerated by every patient, and most will need to allow their gut microbiota to adjust to increasing levels. Gas, bloating and related GI discomfort often accompanies the increased intake of fermentable fiber and can be modulated by reducing the dose or the addition of probiotics. Also, patients who have been prescribed certain carbohydrate restrictions, such as the low FODMAPs diet, should refrain from consuming most prebiotic fibers (See low FODMAPs diet on page 12).

Supplementing Prebiotics

There are numerous products designed to deliver one or more prebiotic available to consumers worldwide. These products usually deliver several grams of prebiotic fiber in ready-to-drink beverages, powders, bars or other functional foods. Products combining prebiotic fibers with probiotic organisms are called "synbiotics." Since the probiotic must remain inert prior to ingestion, there is no particular benefit to delivering both a prebiotic and a probiotic in the same product, with the exception of convenience. Manufacturers not careful to ensure fibers mixed with probiotics have been specially prepared to maintain a low water activity may inadvertently reduce the viability/shelf life of a probiotic by introducing moisture during the manufacturing process. Here are the most commonly used "traditional" prebiotic ingredients.

Inulin-Type Fructans[27]

Inulin-Type Fructans describe a class of linear fructan compounds which includes native inulin, fructooligosaccharides (FOS), and oligofructose. Oligofructose is a shorter-chain inulin extracted from plants and can also be produced by partial enzymatic hydrolysis of inulin. FOS can be manufactured from sucrose and fructose by an enzymatic process or may also be extracted from plants. Native inulin and oligofructose are found in artichokes, asparagus, bananas, chicory root, garlic, onions, leeks, and wheat. However, most commercially available inulin-like fructans are synthesized from sucrose or extracted from chicory roots, Jerusalem artichoke, or agave. The longer chain length of inulin reduces its solubility but gives it a creamy

texture, lending itself to function as a fat replacement in spreads, baked goods, dairy products, frozen desserts, and dressings. The shorter chain length of FOS increases its solubility. This gives FOS properties similar to those of sugar, though they provide ~30–50% of the sweetness of table sugar and are commonly used in cereals, fruit yogurts, frozen desserts, and cookies.

Inulin and FOS have been investigated for a wide-range of prebiotic activities in animals and humans.[28] As a fiber, inulin has been used to increase the frequency of bowel movements in constipated patients; and in 2015, the European Food Safety Authority agreed that 12 grams/day of inulin contributes to maintenance of normal defecation by increasing stool frequency.[29,30] In a recent systematic review of inulin's reported prebiotic activities, inulin supplementation appears to stimulate the growth of several *Bifidobacterium* species, though some studies also show an increase in other genera such as *Faecalibacterium* or *Lactobaccilus*.[31] Interestingly, this systematic review did not show consistent increases in short-chain fatty acid (SCFA) production after inulin supplementation, one of the putative benefits of inulin's bifidogenic activities. Some studies suggested that a higher baseline *Bacteroides/Bifidobacterium* ratio or particular measures of microbiome diversity may result in a higher post-inulin SCFA production (though FOS proved to be a better substrate than inulin in some groups).[32,33]

Other Oligosaccharides (GOS, XOS, MOS)

Fermentable oligosaccharides from other sugar monomers besides fructose have also been shown to have prebiotic activity. The primary ones found in commercially-available products are galacto-oligosaccharides (GOS), xylo-oligosaccharide (XOS), manno-oligosaccharides (MOS), though several others have been developed. As with FOS, many of these compounds have

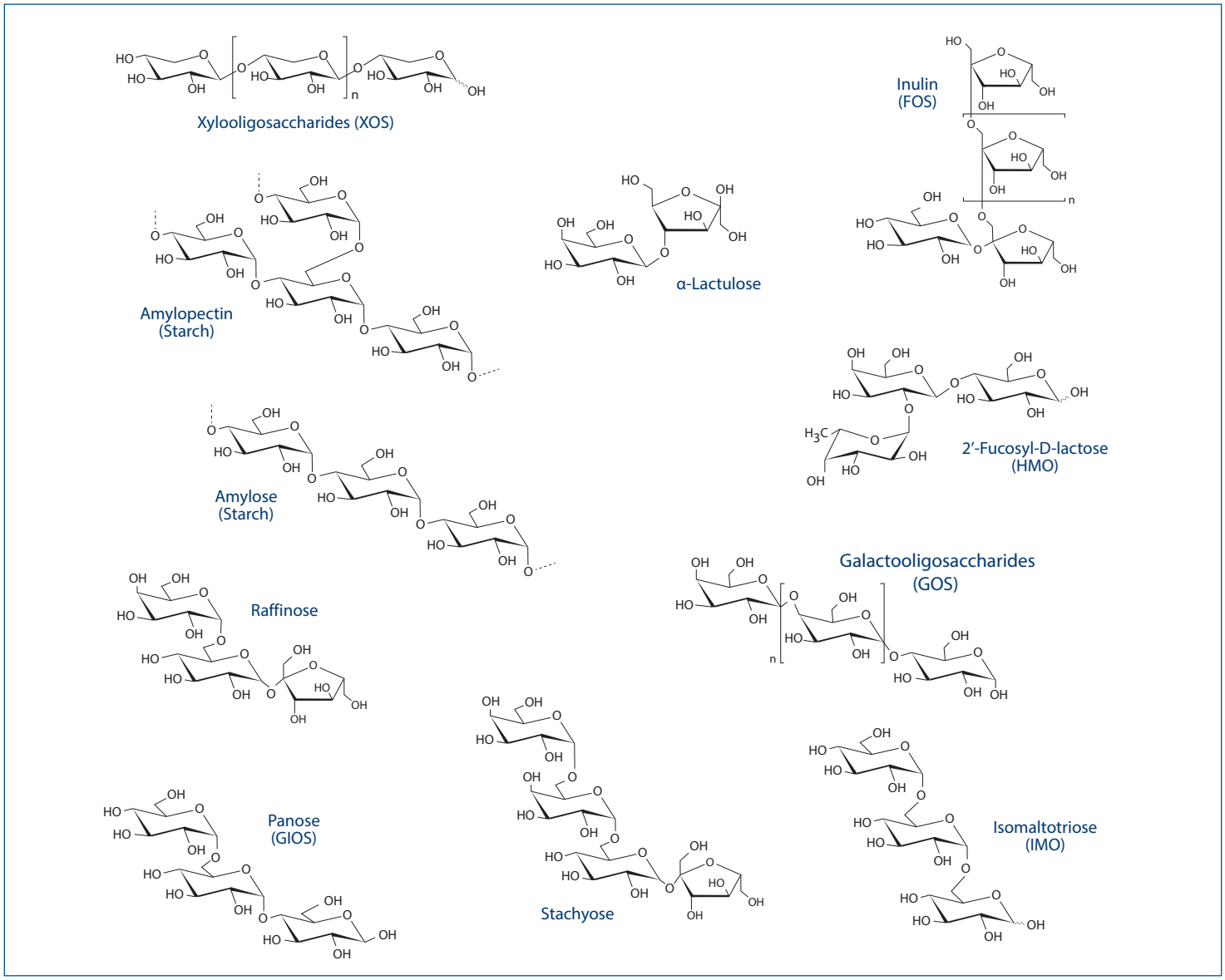

Figure A: Molecular Structure of Common Prebiotics. Figure adapted from AIMS Microbiology, 2015, 1(1): 48-71.

been clinically investigated in both healthy subjects and subjects with various dysfunctions. GOS supplementation has been shown to increase *Bifidobacterium* in adults and children and is often used to supplement pregnant and nursing women, or infants.[34] One study using maternal supplementation of 14.2 g per day of a 9:1 ratio of GOS/FOS was found to favorably modify both the maternal and the developing infant gut microbiome, including increases in acetate production, suggesting that it is possible to modify the development of the infant microbiome by dietary modulation of the maternal gut microbiome.[35] Though less commonly used, XOS and MOS have been shown to have prebiotic effects when given to human subjects, and in some cases, show measurable increases in SCFA production after consumption.[36-40]

Gums, Pectins, Glucans and Related Compounds

The oligosaccharides mentioned above are often extracted from or are similar to the complex oligosaccharides found as gums, pectins and glucans in a variety of plants, fruits and seeds. Since these compounds are often a mixture of fermentable and non-fermentable compounds, their prebiotic effect is not always comparable (dosage-wise) with the purified oligosaccharides that they contain. Nonetheless, prebiotic and fiber formulas often contain a combination of ingredients that include these compounds from a variety of sources. These might be labeled as β-glucans, glucomannan (Konjac), guar gum, Acacia gum/fiber, Gum Arabic, arabinogalactans, and pectin from a number of sources (e.g., apple, okra). In fact, there is an emerging practice of using side-stream of food manufacturing (i.e., waste products) to discover novel fermentable prebiotics, such that fruit peels, husks or seeds that had been routinely thrown away in the past are being investigated for their potential prebiotic (or other health-related) capacity.[41] This includes resistant starches (from potatoes and other sources-see Sidebar), coconut and other nut shells and husks, onion skins, carrot peels, soybean wastewater, sugar beet pulp, cabbage stalks, baobab fruit pulp, seaweed, kiwi fiber and many more. While research with most of these compounds is still limited in humans, *in vitro* and *in silico* studies suggest that some of these compounds may have an ability to preferentially stimulate specific types of gut microbes, allowing for targeted prebiotic effects; especially of non-*Bifidobacterium* keystone species such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii,* specific *Bacteroides* spp, and others.

Host-derived Prebiotics: Human Milk Oligosaccharides and Mucin

The first prebiotic that most humans consume are found in breast milk, which are human milk oligosaccharides (HMOs).[42] Human breast milk contains an average of 5–15 g of oligosaccharides per liter (highest in colostrum), making HMOs the third most abundant solid component of breast milk after lactose and lipids.[43] Structurally, there are hundreds of different HMOs, which are branched polymers of mostly glucose, galactose, N-acetylglucosamine, fucose, and sialic acid monomers.[44] The types of HMOs produced in breast milk is dependent on the genetics of the woman, mostly based on enzymes involved in fucosylation, and these differences have been shown to affect the prebiotic and immune-modulating capacity of HMOs.[45,46]

The interest in HMOs began over 100 years ago, as commercialization of breast milk alternatives for infants started to grow at about the same time increased understanding of healthy gut microbes was being discovered.[47] However, only in the past decade have HMOs, most commonly 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT), been added to infant formula.[48] The addition of these two HMOs to infant formula appears to enrich the gut microbiota with *Bifidobacteria* and delay the shift of the microbiome composition toward an adult-like pattern.[49] In general, studies show that the addition of one or more HMOs to infant formula results in a microbiome shift similar to infants given breast milk (compared to formula fed infants without supplemental HMOs).[50,51,52] Finally, while numerous studies have been performed to investigate the role of HMO supplementation in older children and adults for a wide-range of purposes (e.g., safety studies, microbiome analysis, obesity, inflammatory conditions, GI-related disorders), more research is needed to make recommendations for their use beyond infant microbiome development. We should note that commercially-available HMOs are derived from controlled bacterial fermentation of simple carbon sources and are not extracts or concentrates of human milk.[53]

Mucin, produced and secreted by goblet cells, is an important structural component of the mucosal barrier which plays a vital protective role along the lumen of both the large and small intestines. However, as a highly glycosylated glycoprotein, it can also function as an endogenous prebiotic.[54] Like HMOs, mucin glycosylation is composed of complex and branched polymers of mostly *N*-acetylglucosamine (GlcNAc), galactose, fucose, sialic acid, and N-acetylgalactosamine (GalNAc) monomers; the structures and ratios partly determined by each person's genes.[55] These structures can be utilized by various microbes as nutrients, thereby functioning as prebiotics.

Bacteria with specific carbohydrate-active enzymes (CAZymes) are capable of degrading mucin into smaller moieties (monomers, dimers, etc.), where they can use them as a substrate for their own metabolism or release them into the lumen for other organisms to use. Therefore, organisms with mucin-degrading activity not only thrive on mucin themselves, but function as cross-feeders for other organisms. Some of the most well-recognized mucin-degrading organisms include *Akkermansia muciniphila,* various *Bacteroides spp., Bifidobacterium spp., Ruminococcus spp., Clostridium spp.,* and *Paraclostridium spp.*[56] *Akkermansia muciniphila* is an abundant

and prolific mucin-degrading organism, secreting numerous CAZymes capable of hydrolyzing nearly all known mucin structures.[57] Because of its abundance and unique mucin-degrading capacity, *Akkermansia muciniphila* is considered to be a keystone species in humans and is being studied for its association with a wide-variety of host benefits.[58] Ironically, the alkaloid berberine- known for its ability to increase *Akkermansia muciniphila* levels- has been shown to increase mucin secretion by goblet cells, an activity that might be deemed "prebiotic."[59] Researchers are also investigating the prebiotic and health potential of synthetically-derived mucin, which may be commercially available in the future.[60]

Non-Traditional Microbe-Modulators

For some researchers and ingredient marketers, the broadest definition of a "prebiotic" includes a number of molecules that don't meet our earlier definitions, but have some sort of positive modulating effect on various gut microbes. We include some of them here because healthcare professionals will often see these ingredients combined with traditional prebiotics or marketed directly as prebiotics. Additionally, since microbiome modulation is one of the fastest areas of innovation in medical science (and natural product ingredient research), clinicians should be aware of these agents.

Plant Polyphenols

Polyphenols describe a variety of secondary plant metabolites containing one or more phenolic ring and include molecules such as flavonoids, stilbenes, lignans, and phenolic acids. These compounds are responsible for the color in most fruits and berries, and many of the antioxidant and anti-inflammatory properties of these foods are associated with their polyphenolic content.[61] Nearly all polyphenols absorb poorly and most (often >90%) reach the colon unabsorbed where they can be metabolized by the gut microbiota. The conversion of polyphenols to bioactive phenolic acids is likely the primary way that polyphenols affect host biology, but this activity is dependent on the available metabolic capacity of an individual's microbiota (often called their gut metabotype). Though this is an important and growing area of research (and likely the fundamental driver of personalized responses to many phytotherapies), our focus here is on the research showing phytonutrient influences on microbiota abundance or function.[62,63]

The two primary ways in which polyphenols influence the microbiome is as targeted antimicrobial compounds, or by being converted to a metabolite needed for the growth or activity of a particular microbe.[64] Studies have shown the variety of antimicrobial mechanisms by which polyphenols modulate gut microbial communities.[65] For instance, some polyphenols can inhibit bacterial quorum sensing or prevent biofilm formation. Other studies have shown polyphenols can interact with bacterial proteins to inhibit bacterial nucleic acid synthesis, alter cell membrane function and fluidity, or modify cell wall integrity and synthesis. Some polyphenols can even chelate essential metals like iron, copper, and zinc, which are essential to bacteria metabolism. Any combination of these activities that diminishes the growth or metabolism of specific microbes, allows other microbes to increase their competitive advantage in that particular gastrointestinal niche- thus creating an indirect prebiotic effect.

Enzymes, collectively known as polyphenol-associated enzymes (PAZymes) are produced by numerous gut bacteria allowing them to metabolize various polyphenols. Since most polyphenols from plants are ingested as glycosylated molecules, the removal of these sugar moieties to produce the aglycone polyphenol may provide a small amount of direct prebiotic activity. Beyond that, the use of certain polyphenols or their post-metabolic phenolic acid derivatives have been shown to be used metabolically by some species of bacteria.[66] So, while most polyphenols do not meet the strict definition of a prebiotic, some polyphenols that are known to affect human health do so through their influence on the gut microbiota, and many do so through direct or indirect modulation of the gut microbiota in a prebiotic-like fashion.

Bacteriophages (i.e., Phages)

When discussing the relationship between the human host and their gut microbiome, most of our current knowledge and focus has been on the bacteriome; whereas the gut virome is just beginning to emerge as an important component of this relationship. As it turns out, the vast majority of the viruses detected in the human microbiome are bacteriophages- viruses that infect bacteria rather than human cells (sometimes now called the human phageome).[67] By one estimation, one gram of feces contains between 1-10 billion phage particles.[68] While a detailed discussion of phage biology and their influence on commensal (or pathogenic) organisms is beyond the scope of this review, we want to make the clinician aware that phage therapy (with the intent of modulating the gut microbiota, and thus, human health) is an emerging area of research and phage ingredients are already part of various products being sold as dietary supplements, foods, prebiotics or antibiotics.[69,70] Additionally, genetic modification of phages designed to modulate bacterial function or deliver genetic material to gut bacteria is a novel drug strategy which is likely to continue to grow. Currently, the regulatory status of phages is murky at best. Though certain phages have been approved as food additives (for preservation of spoilage), they do not meet the definition of a dietary supplement ingredient and the US FDA regulates phage therapy as a biologic.[71,72] In contrast to this, phage therapy is quite common in some Eastern European countries and is even available as an over-the-counter pharmaceutical product (as a phage antibiotic) in Russia.[73]

The Types and Uses of Resistant Starches†

Starch is the only complex plant-derived polysaccharide that human enzymes can digest and ancestral reliance on starchy foods appears to have greatly influenced human development.[I,II] Resistant starch (RS) is defined as the sum of starch and starch digestion products that are not broken down or absorbed in the small intestine, and pass into the large intestine.[III] Resistant starches are insoluble fibers and can be grouped into 5 different categories: Type 1 – physically inaccessible starch, Type 2 – native, unmodified starch granules, Type 3 – retrograded starch found in cooked then cooled foods, Type 4 – chemically modified starches, and Type 5 – lipid-starch complexes that resist digestion.[IV] Given that starchy foods have a long history of human consumption and RS consumption, especially types 2 and 3, is a consequence of consuming starchy foods, it can be concluded that RS has been an important source of dietary fiber for most of human history. This is not at all surprising considering all we now know of the important gut microbiome-host relationship and the ability of certain gut microbes to utilize RS as a nutrient.

RS type	Description	Food sources	Digestion in small intestine
RS1	Physically protected	Whole- or partly milled grains and seeds, legumes	Slow rate, partial degree, totally digested if properly milled
RS2	Ungelatinized resistant granules with type B crystallinity, slowly hydrolyzed by α-amylase	Raw potatoes, green bananas, some legumes, high amylose corn	Very slow rate, little degree, totally digested when freshly cooked
RS3	Retrograded starch	Cooked and cooled potatoes, bread, cornflakes, food products with repeated moist heat treatment	Slow rate, partial degree, reversible digestion, digestibility improved by reheating
RS4	Chemically modified starches due to cross-linking with chemical reagents	Foods in which modified starches have been used (e.g., breads, cakes)	A result of chemical modification, can resist hydrolysis
RS5	Amylose-lipid complexes	Foods with high amylose content	Can resist digestion

Classification of different types of resistant starch (RS), their sources and digestion rates. Information assembled from: Raigond P, Ezekiel R, Raigond B. Resistant starch in food: a review. *J Sci Food Agric.* 2015;95(10):1968-1978.

There are a number of contemporary foods that contain resistant starch: Green unripe bananas, raw potatoes, tiger nuts, and other tubers, and various beans and related legumes can be good sources of RS type 2.[V] There are also cultivars of corn, wheat, and barley that are rich in amylose, the non-branched starch polymer, and have a much higher gelatinization temperature, allowing flour from these crops to be used in conventional starch foods like tortillas, pasta, and breakfast cereals.[VI] Starchy foods that have been cooked and then cooled are sources of RS type 3. Examples include potato salad, pasta salad, and sushi rice, although the amount of starch undergoing retrogradation and becoming RS depends on the storage temperature and duration.[VII]

While RS consumption was high in ancestral diets, modern estimates suggest the North American diets only provide 4g/day, roughly 10% of ancestral levels.[V,VIII] Based on historical intake and dietary fiber considerations, nutrition researchers have estimated a required daily RS intake of between 15-20g/day.[V,IX] Although many types of RS undergo gelatinization when heated, some forms can effectively replace digestible starch and reduce the glycemic impact of baked goods.[X] The studies which established these relationships utilized high doses of RS because the greater the amount of digestible starch that is replaced with RS, the greater the glycemic blunting effect that occurs.

High amylose maize starch (HAMS) has been extensively studied in the context of glycemic control in both animal and human studies. Though these studies are quite heterogeneous; in general, consumption of large doses (40 g or more per day) of HAMS improves both post-prandial and chronic measures of blood glucose control and insulin sensitivity.[XI] In 2016, the Food and Drug Administration approved a qualified health claim for products containing HAMS based on studies like these stating "High-amylose maize resistant starch, a type of fiber, may reduce the risk of Type 2 diabetes".[XII] However, it should be noted that the insulin sensitizing effects of HAMS can be reproduced in a mouse model in the absence of gut bacteria, suggesting that these results may be due to the insoluble nature of this fiber rather than its purported prebiotic effects.[XIII]

Similar to HAMS, studies of green banana resistant starch normally result in post-prandial and chronic improvements in blood glucose control and insulin sensitivity, but have not successfully described how the gut microbiome contributes to these health benefits.[XIV] This is in contrast to high doses of resistant potato starch (RPS), which promotes improvements in chronic measures of blood glucose control and insulin sensitivity while also increasing levels of *Bifidobacterium* in the gut.[XV,XVI] Despite these benefits, the high doses of starch from simple food preparations (30g/day or more) needed to deliver effective levels of RS in these clinical trials made it difficult for these ingredients to be easily delivered in dietary supplements and functional food formulations.

† I would like to thank Jason Bush Ph.D., the Chief Scientific Officer at MSP Starch Products Inc., for his contribution to this section on resistant starches

Resistant potato starch is the most thoroughly studied RS from a prebiotic perspective, and recent studies have characterized low dosages that are comparable to oligosaccharide prebiotic dosages. Daily consumption of 3.5g and 7g for 4 weeks led to gut microbiome changes that included increases in *Bifidobacterium spp.* and *Akkermansia muciniphila*, and reductions in both constipation- and diarrhea-associated bowel movements.[XVII] While RPS was known to promote the growth of *Bifidobacterium* at higher doses, the increase in *Akkermansia* was unexpected.[XVI] Levels of *Akkermansia* are characteristically low in obese individuals and patients with type 2 diabetes, and supplementing with probiotic or postbiotic (i.e., pasteurized) forms of *Akkermansia* promote metabolic improvements.[XVIII] Similarly, while many prebiotics, especially short chain oligosaccharides, are effective at relieving constipation, reductions in diarrhea-associated bowel movements are typically only associated with non-prebiotic fibers like psyllium.[XIX] Correlation analysis comparing changes in stool form to changes in taxonomic composition after consumption of RPS revealed a relationship between reductions in constipation-associated bowel movement scores and reductions in *Granulicatella*, *Turicibacter*, and *Gammaproteobacteria*.[XX] Consumption of RPS was not associated with increases in gas, bloating, or abdominal discomfort at 3.5g, 7g, or 30g daily doses demonstrating that RPS is a well-tolerated prebiotic.[XV,XX]

RPS-induced changes in the gut microbiome are also seen through measuring gut-derived metabolites. Metabolomic analysis of serum from participants consuming 3.5g/day of RPS identified significant reductions in histamine, which were not related to diamine oxidase activity but rather to improvements in intestinal barrier function.[XXI] A separate investigation revealed that the same participants experienced a 100 µM reduction in circulating free fatty acid levels, which was correlated with changes in microbially-modified bile acids.[XXII] These findings are intriguing and likely connected to the RPS-dependent increases in *Akkermansia* for several reasons. First, *Akkermansia* utilizes host glycans present in mucins secreted from goblet cells in the intestines and does not directly feed on RPS.[XVIII] Given that the reductions in histamine were attributed to improvements in intestinal barrier function, it is reasonable to conclude that increased mucin production followed improved intestinal cell function, and these mucins supported *Akkermansia* growth.[XXI] Second, given that free fatty acid release is suppressed by insulin signaling and *Akkermansia* promotes improvements in blood glucose levels in part via insulin sensitivity, it is likely that increases in *Akkermansia* are responsible for the decreases in free fatty acid levels.[XXII]

As researchers characterize various categories and types of prebiotics found in natural products, renewed interest in understanding how RS exerts effects beyond glycemic control will likely continue to reveal novel mechanisms and benefits. As a prebiotic, analysis of the changes in microbiota composition after RS consumption (mostly the bacteriome, for now) will still be an important tool, but as recent studies have shown, changes in the microbiome may be inadequate to fully explain the physiological benefits associated with this enigmatic dietary fiber. Metabolomic analysis, to examine both how RS affects the host and the activity of the gut microbiota, can build upon insights from changes in the composition of the microbiome and uncover unanticipated physiological benefits.

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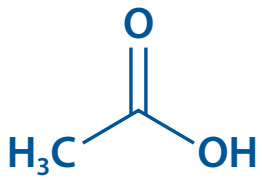
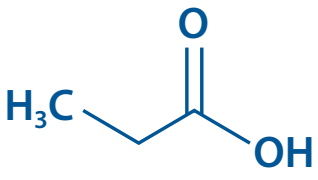
Short-Chain Fatty Acids- A Key Mediator and Biomarker for Prebiotic Activity

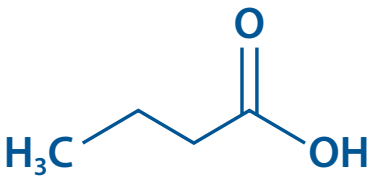
One of the most consistently described features of a healthy microbiome is its ability to produce adequate levels of short-chain fatty acids (SCFAs), primarily butyrate (butyric acid), propionate (propionic acid) and acetate (acetic acid).[74] These two-to-four carbon linear carboxylic acid molecules are produced through the metabolic activities of certain commensal organisms in the gut, when they are given the appropriate substrate (e.g., fermentable fiber). Once formed, SCFAs function in a variety of metabolic and signaling pathways to help maintain the health of the commensal microbes within the gut, as well as a wide-range of systems within the host. Understanding the mechanisms by which SCFAs affect the host will help the clinician realize the importance of GI-derived SCFA production as a clinically-important target for both GI and systemic chronic disease management.



Acetate ($C_2H_4O_2$)



Proprionate ($C_3H_6O_2$)



Butyrate ($C_4H_8O_2$)

The GI Production of SCFAs

The capacity to produce SCFAs from a variety of substrates (i.e., classic prebiotics) through several different pathways resides in a wide-number of bacteria from different phyla and genera.[75] Therefore, the production of SCFAs is dependent on both the amounts and types of SCFA-producing organisms in the gut as well as the availability of fermentable substrates from the diet or through supplementation (though endogenous mucin is also a substrate for SCFA production by certain mucin-degrading organisms like *Akkermansia muciniphila*). Of the primary SCFAs, acetate is the most abundantly produced (~60%), while propionate and butyrate are made in approximately equal amounts (i.e., 20% each). Less abundant are the branched SCFAs such as isovalerate, isobutyrate, and 2-methylbutyrate, which are made from branched-chain amino acids (e.g., leucine, isoleucine, valine), for which much less in known.[76]

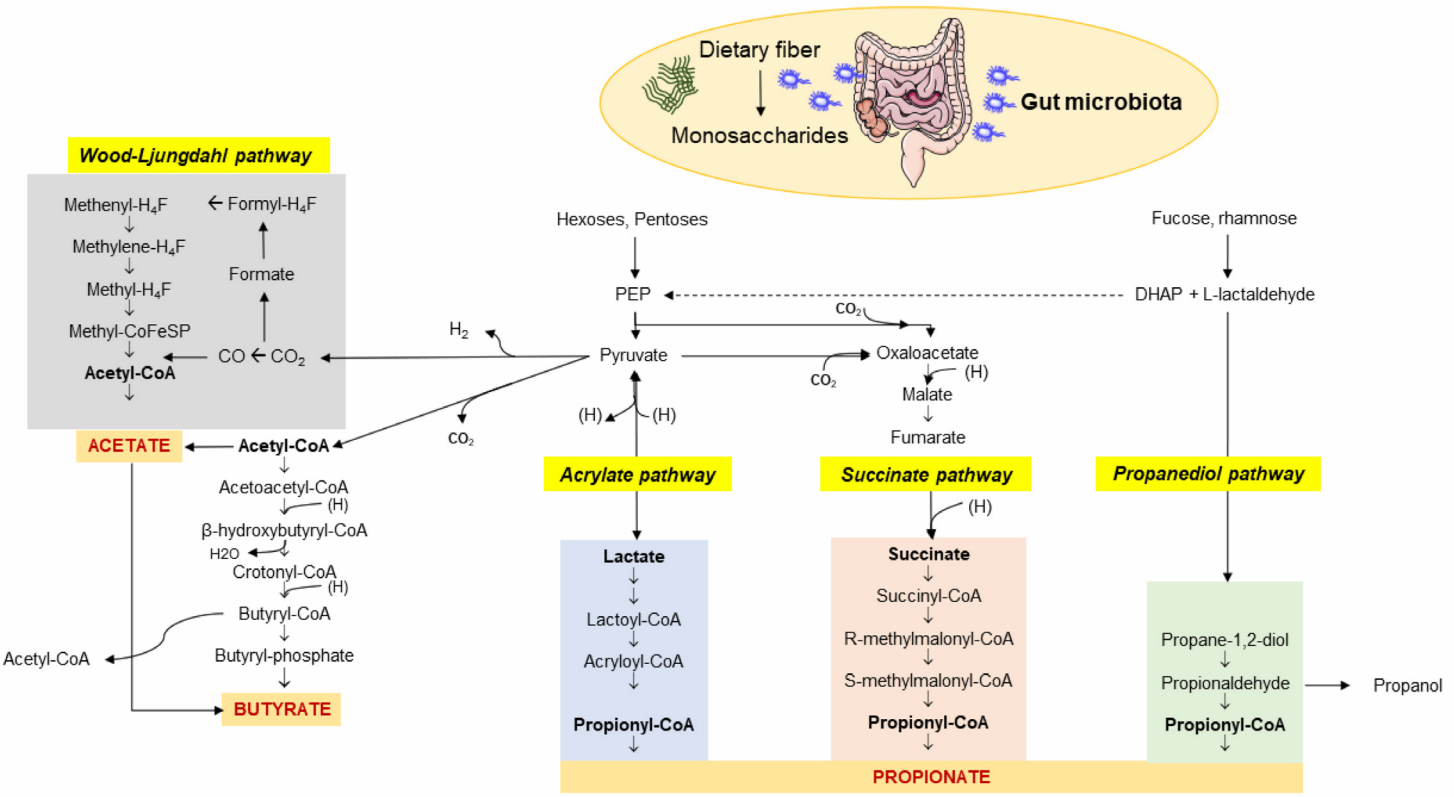
Nearly all of the GI-produced SCFAs (acetate, propionate, and butyrate) are absorbed into gastrointestinal tissues or the bloodstream; less than 5% is excreted in the stool. A large portion of the butyrate produced is absorbed and metabolized by colonocytes whereas both acetate and propionate move to the liver via the portal vein. Since propionate is metabolized in the liver (to propionyl-CoA for use in gluconeogenesis or the citric acid cycle), acetate is the most abundant SCFA in circulation (98-143micromol/L), 20-30 times higher concentration than either propionate (3.8-5.4 micromol/L) and butyrate (0.5-3.3 micromol/L).[77] The abundance and ratio of SCFA in circulation (or stool) can be measured and may, in some contexts, be used to measure health of the GI microbiota or to understand the diet-microbiota health status of an individual.[78,79]

Butyrate as Colonocyte Nutrient

The majority of the butyrate produced in the GI tract, from the conversion of various carbohydrate sources by butyrate-producing microbes, is absorbed in the colon and metabolized by colonocytes, resulting in relatively low concentrations of butyrate in portal blood.[80] Butyrate provides 70-80% of the energy requirements of colonocytes, where it is oxidized to CO_2 in the production of ATP using the mitochondrial oxidative phosphorylation system.[81] This process allows for colonocyte homeostasis, where both the absorption and metabolism of butyrate creates the necessary energy and gradient for the absorption of water and electrolytes in the colon.

Beyond being the source of cellular energy, the oxidation of butyrate by colonocytes generates a hypoxic state which limits oxygen levels in the lumen to help maintain a favorable anaerobic environment for many commensal organisms. This low oxygen state also controls the expression and function of hypoxia-inducible factor (HIF), a transcription factor critical for regulating gut barrier function.[82,83] When butyrate levels are low, colonocytes use glucose as an energy source, shifting from the high oxygen consumption of β-oxidation to anaerobic glycolysis, which consumes much less oxygen. The higher available oxygen drives a decrease in HIF expression, which negatively impacts the epithelial barrier in the gut.

Finally, the absorption and utilization of butyrate by colonocytes also limits butyrate access to stem cells located in the bottom of crypts.[84] Sometimes called the "butyrate paradox," undifferentiated stem cells can actually be inhibited by butyrate availability (likely through inhibition of histone de-acetylation) and thus the utilization of butyrate by colonocytes acts to protect



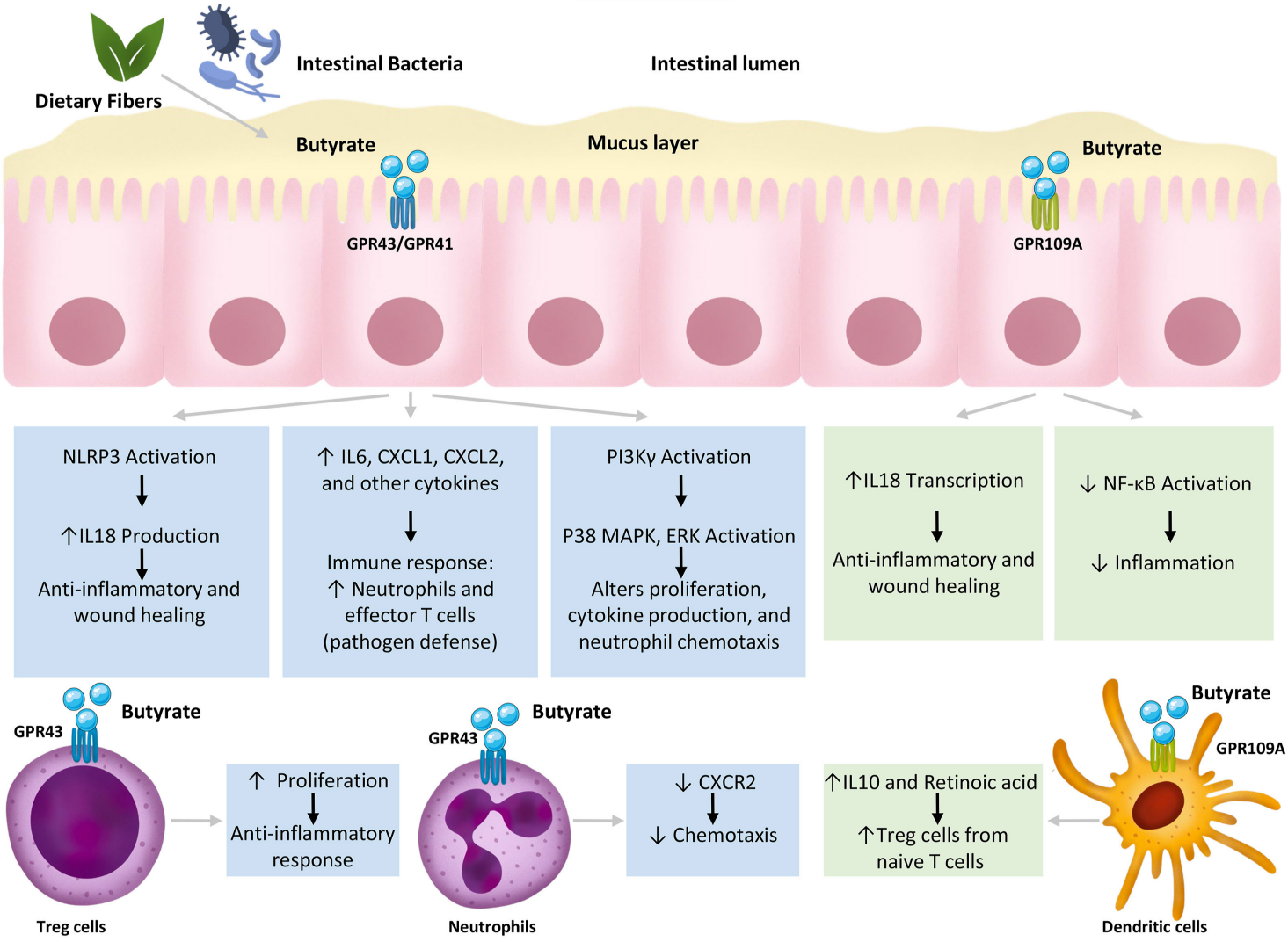
Pathways involved in the biosynthesis of SCFAs from dietary fiber and carbohydrate fermentation by the colonic microbiota. The three major SCFAs are: (1) acetate which originates via the Wood–Ljungdahl pathway or acetyl-CoA; (2) butyrate synthesized from two molecules of acetyl-CoA; (3) propionate from PEP involving the acrylate pathway or the succinate pathway or the propanediol pathway after microbial transformation of fucose and rhamnose. Abbreviations: PEP—phosphoenolpyruvate; DHAP—dihydroxyacetone phosphate. From Portincasa P, Bonfrate L, Vacca M, et al. Gut Microbiota and Short Chain Fatty Acids: Implications in Glucose Homeostasis. *Int J Mol Sci*. 2022;23(3):1105.]

these stem cells.[84] This same phenomenon is thought to explain the role butyrate may play in limiting the growth of cancer cells, which display a similar undifferentiated phenotype that allows butyrate to inhibit their growth.[85]

SCFA as Signaling Molecules in the Gut and Immune System

When prebiotics are consumed resulting in increased levels of SCFA, the benefits extend well beyond the GI system, which is likely due to the fact that these simple fatty acids function as signaling molecules. SCFA bind to several G-protein coupled receptors (GPCRs) found on intestinal, immune, adipose and other tissues leading to metabolic changes in these cells. The receptors GPR43 (also called free fatty acid receptor 2/ FFAR2) and GPR41 (FFAR3) are activated by all three major SCFA. GPR41 is expressed in many tissues, whereas GPR43 is highly specific to lymphatic tissues and various immune cells.[86] Butyrate binds GPR41 preferentially over GPR43, while GPR109A is activated by butyrate and β-hydroxybutyrate.[13] When activated in colonocyte and immune cells, these receptors alter cytokine levels and other downstream signaling pathways to generate an anti-inflammatory immune posture (see figure, page 11). SCFAs also stimulate the production of glucagon-like peptide 1 (GLP-1) and other enteroendocrine signals by binding to GPCRs on L-cells.[87]

Additionally, butyrate can bind to the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) within colonocytes and other cells, which mediates several of the benefits attributed to SCFA. The first was mentioned above, which is that PPARγ activation helps to maintain cells in the metabolic state in which β-oxidation dominates, helping to preserve the hypoxic environment. PPARγ activation is also a critical upstream signal to help reduce inflammatory signaling through NF-κB.[88] Finally, SCFAs (mostly butyrate) have a dose-dependent effect on gene regulation by modulating histone acetylation. That is, at low concentration SCFAs can activate histone acetyltransferases (HATs) and at higher concentrations they inhibit histone deacetylases (HDACs).



Butyrate signaling in the colon through G-protein coupled receptors (GPR41, GPR43, and GPR109A). Butyrate is mainly produced by the intestinal microbiota through the breakdown of dietary fibers. Butyrate signals through three G-protein coupled receptors present on colonic epithelial and immune cells: GPR43 (free fatty acid receptor 2; FFAR2), GPR41 (free fatty acid receptor 3; FFAR3), and GPR109A (hydroxycarboxylic acid receptor 2; HCAR2). Activation of these receptors in colonocyte and immune cells causes changes in cytokine levels and various signaling pathways, promoting an anti-inflammatory response. From: Hodgkinson K, El Abbar F, Dobranowski P, et al. Butyrate's role in human health and the current progress towards its clinical application to treat gastrointestinal disease. *Clin Nutr*. 2023;42(2):61-75. Used under the Creative Commons CC-BY-NC-ND.]

SCFA signals Beyond the Gut

It is now established that metabolites produced in the GI tract influence nearly every tissue of the body, leading to such phenomena as the gut-brain or gut-liver axis. Now it is more common to include the microbiota in this pathway (i.e., the microbiome-gut-brain axis) to acknowledge that many of the signals are derived from the microbiota residing in the gut.[89] SCFA signaling, though GPCRs, modulation of histone acetylation and PPARγ activation are all implicated in most tissues. For instance, several research models suggest that SCFA signaling through these mechanisms improves the integrity of the blood brain barrier (BBB), reduces activation and inflammation in microglial cells and can reduce progression of several neuroinflammatory or neurodegenerative disease models or biomarkers.[90] Similar mechanisms are implicated to explain the benefit of SCFA production for hepatic, adipose and metabolic disorders in many tissues.[91-94]

Limiting Prebiotics: The Low FODMAP Diet

In the larger discussion of the use of prebiotics and fermentable carbohydrates for their ability to modulate the gut microbiota, we acknowledge that some people have a temporary or long-term difficulty in using these compounds. In fact, diets such as the low FODMAP diet have been used to reduce GI symptoms in many patients. Since this strategy can severely limit prebiotic intake, we include this discussion as a balanced way to understand the complexities of symptom-related strategies to promote long-term GI health.

The acronym, FODMAP, stands for **F**ermentable **O**ligosaccharides (e.g., fructans), **D**isaccharides (e.g., lactose), **M**onosaccharides (i.e., fructose) **a**nd **P**olyols (e.g., sorbitol, xylitol, mannitol, etc.). These represent a heterogeneous group of short-chain carbohydrate molecules that are poorly absorbed along the GI tract in certain individuals.[I] Consumption of FODMAPs has been associated with increased luminal distention, leading to symptoms of abdominal bloating, pain and altered bowel habits in certain individuals. These symptoms may lead to the diagnostic signature of IBS or other functional gastrointestinal disorders.[II,III,IV]

There are several proposed mechanisms by which FODMAPs induce these symptoms. First, the absorption of some FODMAP components may be delayed or extremely poor. For instance, disaccharides (e.g., lactose) may be inadequately hydrolyzed in the small intestine by brush border enzymes, or monosaccharides (e.g., fructose in excess of glucose) may have a low absorptive capacity leaving excess FODMAP solutes in the lumen of the small intestine. These solutes create an osmotic potential, forcing water to enter the lumen to maintain osmotic homeostasis, which contributes to abdominal bloating, distention, and pain in those with visceral hypersensitivity. Secondly, because of the slow absorption or malabsorption of FODMAPs, bacteria present within the colonic microbiome readily ferment the substrates into short-chain fatty acids (e.g., butyrate, propionate, acetate) and gases (e.g., hydrogen, carbon dioxide, and methane in some individuals). Thirdly, FODMAPs have been shown to alter motility; fructose-sorbitol ingestion has been shown to reduce orocecal transit time in healthy subjects.[V]

What is the Low FODMAP Diet?

According to Mansueto et al., beginning in the 1980s, researchers observed the consumption of poorly absorbed, short-chain carbohydrates was associated with induction of GI symptoms.[IV] Eventually, in 2005, a research team at Monash University in Australia used the term FODMAP to categorize and characterize these symptom-provoking food components. The group suggested a diet low in FODMAP foods may be of benefit for GI symptom relief in those with functional and organic GI disorders such as IBS and IBD.[VI] Since 2005, research exploring the low FODMAP diet for GI symptom relief has been very active.

Although some studies have been cited as having low methodological quality, partly due to the complexity of dietary intervention studies, most published research has shown benefit for the low FODMAP diet in IBS. (Staudacher et al. #12) [VII,VIII,IX] A systematic review and meta-analysis by Marsh et al. showed benefit for the low FODMAP diet for functional gastrointestinal symptoms including: abdominal pain, bloating, distension, constipation, diarrhea and flatulence.[X] The British Dietetic Association updated their evidence-based guidelines for the dietary management of IBS in adults and included a recommendation for the low FODMAP diet in these patients; additionally, national guidelines from Japan and the United Kingdom recommend the low FODMAP diet in IBS management. [XI,XII,XIII]

Implementation of the Low FODMAP Diet:

Partly owing to risk for nutritional inadequacy following the low FODMAP diet, the complexity of the diet, and the paucity of evidence surrounding self-directed patient administration of the diet, it is widely cited that implementation of the low FODMAP diet should be done under the supervision of a dietitian, nutritionist, or other healthcare provider educated in this diet.[XIV] In most studies, implementation of the low FODMAP diet by a healthcare provider has been in a one-on-one setting where the educator provides individualized dietary advice while ensuring nutritional adequacy within the overall diet (often by providing reference materials to the patient). However, evidence is now emerging for greater cost benefits and clinical effectiveness of the use of group-based low FODMAP dietary education and implementation.[XV]

Essentially, the low FODMAP diet is an "elimination and re-challenge" diet, where patients identify foods in their current diet that are high in FODMAPs and replace them with alternatives low in FODMAPs (see Table 1 for list[XVI]). The elimination portion of the diet begins with the "induction phase" where the patient adheres to a stringent and restricted

diet eliminating all high FODMAP foods for four to six weeks.[XVII] It should be noted that the length of the "induction phase" is not defined by the evidence from clinical trials. In fact, many clinical studies report subjects experience symptom reduction after about seven days; however, De Giorgio et al. explain that an extended induction period (i.e., four to six weeks) is proposed to allow the patient time to learn the diet and ensure symptomatic persistence of the effect.[I]

The status of symptoms at the end of the "induction phase" determines whether or not a re-challenge phase is necessary. If an adherent patient's GI symptoms are not improved following the "induction phase," the patient should discontinue the low FODMAP diet and seek other dietary or appropriate therapies. However, if the patient experiences symptom improvement after eliminating foods high in FODMAPs, then the patient is advised to follow an individualized, "step-down" food reintroduction plan to determine tolerance of certain FODMAP-containing foods. Different groups of FODMAPs (i.e., monosaccharides, polyols, oligosaccharides) may have different osmotic and fermentative potential based largely on their molecular weight and degree/rate of absorption, resulting in heterogeneity among different FODMAP components. At the same time, there may be heterogeneity in individual response to different FODMAP components or FODMAP-containing foods across individuals. The goal of the reintroduction, or re-challenge, phase is to diversify and minimize unnecessary dietary restriction for an individual as much as possible, and restrict only to the level needed for symptomatic control[XVIII]

Limitations of the Low FODMAPs Diet:

TYPES OF SUGARS	HIGH FODMAP FOODS	LOW FODMAP ALTERNATIVES
Oligosaccharides	**Fruits:** Watermelon, white peaches, persimmon, prunes, nectarines and most dried fruit	**Fruits:** Banana, most berries (except boysenberries and blackberries), grapes, lemon juice, lime juice, mandarin, orange, kiwifruit, pineapple, passion fruit, and rhubarb
	Vegetables: Onion, garlic, artichokes, leeks, beetroot, savoy cabbage and peas (except sugar-snap peas)	**Vegetables:** Capsicum, bok choy, green beans, parsnip, silverbeet, cucumber, carrots, celery, eggplant, lettuce, potatoes, yams, tomatoes, and zucchini
	Legumes: Red kidney beans (boiled), baked beans, and soya beans (boiled)	
	Grains: Wheat-, rye-, and barley-based products	**Grains:** Wheat-free grains/flour, gluten-free bread or cereal products, and quinoa
	Fibers: FOS and GOS	
Disaccharides	**Lactose:** Dairy products: cows/goat milk, and yogurt	**Lactose-free**: Almond or rice-based milk, yogurt and ice cream, hard cheese, feta and cottage cheese
Monosaccharides	**Fruits:** Apples, pears, watermelon, mango, cherries, boysenberries and fruit juice from high-fructose foods	**Fruits:** Banana, grapes, honeydew melon, kiwifruit, lemon juice, lime juice, mandarin, orange, passion fruit, paw paw, and most berries (except boysenberries and blackberries)
	Vegetables: Asparagus and sugar-snap peas	**Vegetables:** Green beans, broccoli, Brussels sprouts, carrots, eggplant
	Sweeteners: Honey, high-fructose corn syrup , fructose (in excess of glucose)	**Sweeteners:** Maple syrup
Polyols	**Fruits:** Apples, pears, avocado, apricots, blackberries, nectarines, peaches, plums, prunes, and watermelon	**Fruits:** Banana, grapes, honeydew melon, kiwifruit, lemon juice, lime juice, mandarin, orange, passion fruit, and paw paw
	Vegetables: Sweet potato, mushrooms, cauliflower, and snow peas	**Vegetables:** Green beans, broccoli, Brussels sprouts, carrots, eggplant
	Sweeteners: Mannitol and Sorbitol	**Sweeteners:** Maple syrup, and sugar (sucrose)

Table 1: Basic list of Foods high or low in FODMAPs. Modified from Nanayakkara WS, Skidmore PM, O'Brien L, et al. Efficacy of the low FODMAP diet for treating irritable bowel syndrome: the evidence to date. *Clin Exp Gastroenterol*. 2016 Jun 17;9:131-42, (licensed under a Creative Commons Attribution 3.0 License) and Monash University. Low FODMAP Diet Application.

One of the most commonly cited limitations of the low FODMAP diet is the unknown effects that long-term adherence to the diet may pose on the microbiome, as many "prebiotic fibers" would be limited when following a low FODMAPs diet. Staudacher et al. showed the low FODMAP diet was able to better manage IBS symptoms after four weeks of fermentable carbohydrate restriction; however, the low FODMAP diet was also associated with a decreased abundance of *Bifidobacteria*.[XIX] The group also found calcium intake was lower in the low FODMAP diet group ($p=0.012$), which may have been due to reduction in food selection (e.g., dairy intake). Another more recent trial found a low FODMAP diet (3.05 g/day FODMAPs content) was associated with a significant reduction in total bacterial abundance ($p<0.001$) but significantly increased bacterial diversity ($p<0.001$) compared to a typical Australian diet (23.7 g/day FODMAPs content).[XX] Other limitations include the perceived difficulty of adhering to the diet, and the nutritional adequacy of the

diet (e.g., calcium intake). Based on these limitations, especially the unknown effects that low FODMAP diets may pose on an individual's microbiome, this diet is not recommended to the general population without GI symptoms.[XXI,XXII] For symptomatic individuals, it is recommended to restrict the diet only to the level of symptom control, reintroducing as many components as symptomatically feasible.

Additional information regarding the low FODMAP diet and FODMAP foods is available on the Monash University website; the group also has created a mobile application called the "Monash University Low FODMAP Diet App."[XXIII]

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Summary and Principles for Using Prebiotics

- Prebiotics are an important therapeutic category of ingredients to help modulate the gut microbiota. Since they modulate whole classes of commensal organisms, they are often more foundational for gut health that probiotics or postbiotics.
- No one prebiotic type or source is complete enough to promote all the necessary commensal organisms needed for the health of the gut microbiome or host. Prebiotic products should include several different types and sources of prebiotics (especially those that are also considered dietary fibers or resistant starches), as well as additional non-traditional microbe modulators like phytonutrients or mucin-stimulating ingredients (e.g., berberine).
- Introduce prebiotics and fermentable fibers slowly, and gradually increase the dose to the desired target level. Note that some ingredients will require over 10 grams/day to facilitate their benefit, while others may show benefits at doses less than 1 gram per day (mostly polyphenols).
- Common side-effects when starting prebiotics may include gas, bloating and changes in bowel function. These are dose-dependent and temporary for most people and are evidence of their microbiome-altering effects [See Sidebar on low FODMAP diet for those who cannot tolerate fermentable carbohydrates].
- New prebiotic sources and compounds are being discovered and investigated for specific clinical benefits on a regular basis- this is an emerging area of innovation. While newly discovered ingredients may not always outperform older ones, be prepared to try new prebiotic sources and combinations of products to help a wide-range of patient needs.

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