Review Article

Inulin-Type Prebiotics – A Review: Part 1

Greg Kelly, ND

Abstract

This article is part 1 of a two-part review of inulin-type prebiotics. Prebiotics are a category of nutritional compounds grouped together by the ability to promote the growth of specific beneficial (probiotic) gut bacteria. Inulin-type prebiotics contain fructans of the inulin-type. Fructans are a category of nutritional compounds that encompasses naturally occurring plant oligo- and polysaccharides in which one or more fructosyl-fructose linkages comprise the majority of glycosidic bonds. To be "inulin-type" a fructan must have beta (2–1) fructosyl-fructose glycosidic bonds, which gives inulin its unique structural and physiological properties, allowing it to resist enzymatic hydrolysis by human salivary and small intestinal digestive enzymes. Inulin-type prebiotics include fructooligosaccharides (FOS), oligofructose, and inulin – terms that have been used inconsistently in both the scientific literature and in food applications. Commercially available inulin-type prebiotics can be extracted from food (typically chicory root) or synthesized from a more fundamental molecule (typically sucrose). Depending on the starting source and degree of processing, inulin-type prebiotics can be produced with very different chemical compositions. Some inulin-type prebiotics are relatively high in free sugars (the monosaccharides fructose and glucose and the disaccharide sucrose), while others have most or all free sugars removed. Processing can also result in mixes consisting exclusively of inulin-type oligosaccharides, polysaccharides, or both. Because inulin, oligofructose, and FOS resist enzymatic digestion in the upper gastrointestinal tract, they reach the colon virtually intact where they undergo bacterial fermentation. All inulin-type prebiotics are bifidogenic – stimulating the growth of Bifidobacteria species. The effects they have on other gut organisms are less consistent. A minimal dose of inulin-type prebiotic appears to be needed to produce a bifidogenic effect. However, intraindividual response to an identical dose of the same inulin-type prebiotic, in terms of stimulation of total number of Bifidobacteria and individual Bifidobacteria species, can be variable. Research on therapeutic uses of inulin-type prebiotics will be covered in part 2 of this review. (*Altern Med Rev* 2008;13(4):315-329)

Introduction

Prebiotics are a category of nutritional compounds grouped together, not necessarily by structural similarities, but by ability to promote the growth of specific beneficial (probiotic) gut bacteria. Many dietary fibers, especially soluble fibers, exhibit some prebiotic activity; however, non-fiber compounds are not precluded from being classified as prebiotics presuming they meet the requisite functional criteria.

Gibson and Roberfroid offered a definition of prebiotics in a 1995 introductory article. They defined prebiotics as "non-digestible substances that when consumed provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria."[1] Roberfroid updated this definition in a 2007 review

Gregory Kelly, ND – Founding partner of Direct Access eHealth; Contributing Editor, *Alternative Medicine Review*; past instructor, University of Bridgeport in the College of Naturopathic Medicine; published articles on various aspects of natural medicine and contributed three chapters to the *Textbook of Natural Medicine*, 2nd edition; teaches courses on weight management, the role of stress in health and disease, chronobiology of performance and health, and mind-body medicine.
E-mail: greghealthcoach@netscape.net

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article on prebiotics: "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health."[2]

Definitions of prebiotics typically have in common an emphasis on the compound being non-digestible (and hence subject to colonic enzymatic activity and fermentation by colonic bacteria) and able to selectively stimulate the growth of one or more desirable or health-enhancing types of gut bacteria. While definitions of prebiotics do not emphasize a specific bacterial group, the number and/or activity of Bifidobacteria and other lactic acid-producing bacteria must be increased for the compound to qualify as a prebiotic. Either implicitly or explicitly within most definitions is the concept that the compound improve the health of the subject consuming it.

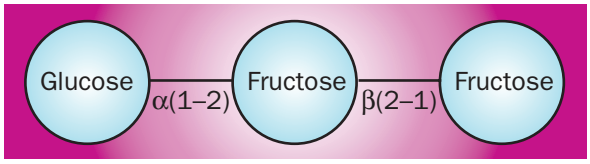
While many nutritional compounds have some degree of prebiotic activity, Roberfroid identified two groupings of nutritional compounds that met his definition, inulin-type prebiotics and galactooligosaccharides (GOS).[2]

Other nutritional compounds suggested as prebiotics, but not included by Roberfroid as prebiotics, include gentiooligosaccharides, glucooligosaccharides, isomaltooligosaccharides, mannan oligosaccharides, N-acetylchitooligosaccharides, oligosaccharides from melibiose, pectic oligosaccharides, xylooligosaccharides, gums (like gum Arabic), hemicellulose-rich substrates, resistant starches (such as resistant maltodextrin), lactosucrose, oligodextrans, polydextrose, germinated barley, gluconic acid, glutamine, lactose, and the simple sugar tagatose (a mirror image of fructose).[2-5] Because research on several of these compounds for prebiotic activity is promising, it is possible that in the future one or more of these compounds might also meet the criteria specified in Roberfroid's definition of prebiotics.

This review article will focus on one of the two subcategories of prebiotics Roberfroid included in his definition – inulin-type prebiotics. This grouping was selected because they represent the most widely commercially-available prebiotic compounds. Part 1 of this review defines inulin-type prebiotics, explores food applications, and examines their ability to modulate gut microflora. In part 2, studies examining the use of inulin-type prebiotics as a therapeutic option will be reviewed.

Inulin-type prebiotics include inulin, oligofructose, and fructooligosaccharides (FOS), oligo- or

Figure 1. GF_2 Fructan



polysaccharide chains comprised primarily of linked fructose molecules that are bifidogenic. Inulin-type prebiotics are used as functional food ingredients in beverages, yogurts, biscuits, and spreads; they are also used as dietary supplements.

Inulin-type prebiotic compounds are naturally occurring constituents in many plants. Root vegetables including Jerusalem artichokes, burdock, chicory, leeks, and onions are especially rich sources. Estimates suggest a person consuming an average North American diet ingests 1-4 g inulin-type prebiotic compounds daily, a low daily intake compared to a less processed diet, higher in plant foods. It is also considered low compared to other regions of the world, such as Europe, where estimates suggest an average daily intake of 3-11 g.[6]

General Chemical Structure of Inulin-type Prebiotics

Inulin-type prebiotics are members of a larger group called "fructans." Fructans represent a category of compounds that encompasses all naturally occurring plant oligo- and polysaccharides in which one or more fructosyl-fructose linkages comprise the majority of glycosidic bonds; hence, they are primarily polymers of fructose units. Fructans can have at least one fructosyl-glucose linkage – identical to that found with sucrose and, when present, is typically a starting link in the polymer chain. When the starting molecule is sucrose in the fructan chain, the bond between the starting glucose and the second carbon (of fructose) can be hydrolyzed to some degree by sucrase enzymes, secreted by the tips of the small intestinal epithelial villi, and produce free glucose. The presence of this sucrose sugar moiety is not a necessary precondition for the compound to be considered a fructan; therefore, many fructans begin with fructose. Structurally, fructans can be linear or branched fructose polymers.

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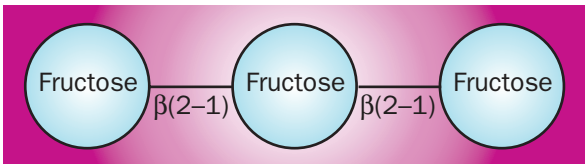
Figure 2. F_3 Fructan



An individual fructan having a glucose molecule preceding fructose is designated as GF_n – G referring to the terminal glucose unit, F referring to fructose units, and n designating the number of fructose units found in the fructan chain. Hence, GF_2 is a fructan oligosaccharide with a terminal glucose followed by two fructose units (Figure 1). This fructan has one fructosyl-glucose linkage (a sucrose molecule) followed by one fructosyl-fructose linkage. A fructan with no glucose would be designated as either F_n or F_m. Both are used in the scientific literature with n (or m) referring to the number of fructose units occurring in the fructan. In this review, n will be used. A fructan designated as F_3 (Figure 2) has three fructose units and two fructosyl-fructose linkages.

Fructans can also be described by degree of polymerization (DP). DP refers to the number of repeat units in an oligomer or polymer chain, so DP of an individual fructan would be its number of repeating fructose units and identical to n.

Inulin is a generic term that covers all linear fructans with beta (2–1) fructosyl-fructose glycosidic bonds.[2] This specific type of glycosidic bond gives inulin its unique structural and physiological properties. Because of the beta configuration of the bonds between fructose monomers, inulin-type fructans resist enzymatic hydrolysis by human salivary and small intestinal digestive enzymes – specific for alpha-glycosidic bonds. As a result, inulin-type fructans are indigestible and are fermented in the colon.[2]

General Description of Commercial Inulin-type Prebiotics

Although commercially available inulin-type prebiotics meet the general chemical structure needed to be called inulin-type, they are not comprised homogenously of a single type of fructan. Inulin-type prebiotics can be extracted from a food source (typically chicory root) or synthesized from a more fundamental molecule (typically sucrose). In either instance, the resultant inulin-type prebiotic consists of mixtures of inulin-type fructan molecules with varying DP. Some inulin-type fructans begin with glucose as the starting unit (GF_n type fructans), while others do not include a glucose monosaccharide unit (F_n type fructans). Some inulin-type prebiotic products have a low proportion of glucose units, while others have far higher proportions. Within the overall mixture there might be inulin-type fructans that consist of as few as two fructose units or more than 60 fructose units. Despite these variations, as long as the fructans in the product are of the inulin-type, the product is correctly classified as an inulin-type prebiotic.

Commercial products comprised of mixtures of inulin-type fructans with varying DP are commonly described by the average (DPav), maximum (DPmax) value, and/or range of DP values found in the product. Commercially available inulin-type prebiotic products consist of a variety of chains with varying DP values.

For example, inulin extracted from chicory by hot water extraction would have inulin-type fructans that vary in the number of fructose units from a low of two to a maximum of 60. This relatively unprocessed inulin has a DPav of about 12, a DPmax of 60, and a DP range of 2-60. Approximately 10 percent of the fructan chains have a DP ranging from 2-4. Some of the fructans start with glucose (GF_n type) and others with fructose (F_n type).[2] Conversely, a fructan-type prebiotic synthesized from sucrose has a very different DP portfolio; all the fructans start with glucose (GF_n type). A synthesized fructan-type prebiotic might have a DPav of 3.6, a DPmax of 4, and a DP range from 2-4.[2] While these would both be correctly labeled inulin-type prebiotics, they vary considerably in terms of actual fructans found in the product.

Generic Nomenclature of Inulin-type Prebiotics

There is no uniform standard for naming inulin-type prebiotics. The three generic terms encountered most frequently are inulin, oligofructose, and FOS, and these terms are not used in the same way in research articles.

In order to understand the generic terms used for inulin-type prebiotics, it is useful to note that inulin-type fructans can be broken down into broad

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subgroups based on DP. Inulin-type fructans consisting of DP ≥10 are considered long chain (high-molecular weight), while inulin-type fructans with a DP <10 are considered short chain (low-molecular weight). In some instances, reviews of this topic further subdivide the group with a DP of less than 10 into short-chain (DP of 2-4) and medium chain (DP of 5-9) groupings. While there appears to be consistency in what is considered long chain, the same consistency is not found in short-chain nomenclature. In large part, the generic names inulin, oligofructose, and FOS reflect these DP-based subgroupings; however, there is a great degree of inconsistency in how they have been used.

Inconsistency in the Use of the Term FOS

Some experts in the field, including Roberfroid, consider oligofructose and FOS as synonymous terms for inulin-type fructan mixtures, as long as the fructan is chemically of the inulin-type and the DPmax is <10. Despite recognition that these names might be used interchangeably, Roberfroid refers to inulin-type prebiotics that meet the above criteria as oligofructose. He uses the term inulin as a generic description of inulin-type fructan mixtures that contain at least some fructan chains with DP ≥10 and uses the term "inulin HP" to describe mixes that consist entirely of inulin-type fructans with a high-molecular weight – DP ≥10.[7]

These nomenclature standards differ from the terminology Roberfroid used in a 1995 introduction to the topic co-authored by Gibson. In that paper FOS was used to encompass both oligofructose and inulin;[1] it was used as a synonym for the entire category of inulin-type prebiotics, resulting in some confusion.

In addition, other authors do not use the same naming standards as Roberfroid. Carabin and Flamm, in their review of the safety of inulin and oligofructose, use FOS to describe mixtures of short-chain inulin-type prebiotics synthesized from sucrose. These mixtures include only short-chain inulin-type fructans that have the GF_n chemical structure and to which 1-3 additional fructose units have been enzymatically added to the fructose residue of sucrose (DPmax 4). They use the term oligofructose for mixes produced by partial hydrolysis of inulin. These mixes contain both GF_n and F_n type fructans and consist exclusively of fructans with a DPmax of <10. They use the generic term inulin for mixes produced from inulin that have any fructans with a DPmax ≥10; they do not provide terminology for mixes that consist entirely of fructans with DP ≥10.[8]

As is apparent, terms Roberfroid uses interchangeably – oligofructose and FOS – Carrabin and Flamm use to refer to two different production techniques that result in different mixes of inulin-type fructans. Coussement appears to suggest the convention adopted by Carrabin and Flamm is used more frequently in the literature when he states: "In most cases, oligofructose refers to the partial hydrolysate of inulin."[9]

The potential for confusion caused by inconsistent nomenclature is evident when reviewing research. As an example, Alles et al use the generic term FOS in the title of their paper.[10] In the methods section of the study, the specific material was characterized as an inulin-type prebiotic made by partial hydrolysis of inulin. Carrabin and Flamm would categorize this as oligofructose; it would not meet their criteria for FOS. Roberfroid would also call it oligofructose, but would recognize FOS as synonymous. This potentially confusing use of FOS and oligofructose occurs periodically in the literature. Although Euler et al[11] and Oleson et al[12] use the generic term FOS in the title of their papers, what they actually used was produced by partial hydrolysis of inulin from chicory root. To add to the inconsistency, Bouhnik et al[13] and other authors[14-17] use the term short-chain FOS (scFOS) for inulin-type prebiotics synthesized from sucrose.

Inconsistency in the Use of the Term Inulin

The generic term inulin is also used inconsistently. In its most generic sense inulin is a term that covers all fructans of the inulin-type.[2] Roberfroid specifically uses inulin as the generic term to describe the hot water extract of chicory root and the term inulin HP to describe a mix that has undergone physical separation techniques to remove all but long-chain, high-molecular weight inulin.[7] Carabin and Flamm do not differentiate between a hot-water extract and a product processed to contain only long-chain fructans.[8] Other authors use the term "native inulin" to describe the hot-water extract.[9,18] Some authors use the term long-chain FOS (lcFOS) for the long-chain, high-molecular weight fraction of inulin extracted from chicory root (what Roberfroid describes as inulin HP).[19,20] Thus, inulin might be used generically or specifically. Two articles highlight this point.[21,22] Both contain the term "inulin" in the title, but according to the

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Table 1. Inulin-Type Prebiotic Nomenclature

FOS	This term will be used to describe short-chain, inulin-type fructan mixes synthesized from sucrose.
Oligofructose	This term will be used to describe inulin-type fructan mixes with a DPmax <10 that have been produced by partial hydrolysis of inulin and then undergone physical separation to remove all long chain (DP ≥10) inulin-type frutans.
Inulin	This term will be used to describe the hot water extracts that result in inulin fructans that have not undergone further processing.
Inulin HP	This term will be used to describe the exclusively long-chain, high-molecular weight mixes of inulin-type fructans (fructans with a DP <10 physically removed).
FOS-enriched inulin	This term will be used to describe proprietary mixes that enrich inulin with FOS.
FOS-enriched inulin HP	This term will be used to describe proprietary mixes that enrich inulin HP with FOS.
Oligofructose-enriched inulin	This term will be used to describe proprietary mixes that enrich inulin with oligofructose.
Oligofructose-enriched inulin HP	This term will be used to describe proprietary mixes that enrich inulin HP with oligofructose.

methods section what they are referring to in both cases is what Roberfroid defines as inulin HP.

Inconsistency in the Naming of Proprietary Mixes

Some researchers appear to have their own nomenclature standards. For example, Lindsay et al describe their prebiotic as FOS,[23] a mixture of 70-percent oligofructose and 30-percent inulin – which is neither the scFOS from sucrose of Carabin and Flamm nor the synonym for oligofructose of Roberfroid. It is actually a proprietary mix processed to yield a specific ratio of oligofructose and inulin. One possible name for such mixes is oligofructose-enriched inulin. Unfortunately, there is no agreed upon nomenclature to describe these proprietary mixes produced by concentrating one or more categories of inulin-type fructans based on specific DP.

Nomenclature in this Review

Because there are no uniformly accepted standards of nomenclature for inulin-type prebiotics, this article uses nomenclature outlined in Table 1.

In instances where the authors of a research paper specify what nomenclature they used, the above nomenclature will be used even if the original paper used different terminology.

Production of Inulin-type Prebiotics

Production of the inulin-type prebiotics oligofructose, inulin, and inulin HP begins with a plant

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source rich in fructans. Production of FOS begins with a more fundamental molecule (typically sucrose). Commercially available inulin-type prebiotics differ in purity, fructan and free-sugar content, and fructan portfolios. By applying specific production technologies to either a food source of fructans (such as chicory root) or sucrose, the food industry can produce a diversity of inulin-type prebiotic products with different DP and sugar portfolios with slightly different strengths and weaknesses.

Production of Oligofructose, Inulin, and Inulin HP

The starting plant material for production of oligofructose, inulin, and inulin HP is in most cases chicory root (Figure 3), preferred because it contains high amounts of inulin-type fructans. The inulin is extracted by hot water. The result of this extraction is ~92-percent inulin-type fructans of both GF_n and F_n types with DP ranging from 2-60 and a DPav of ~10-12. About 10 percent of the fructans in this minimally processed inulin might have a DP ranging from 2-4 and 20 percent might range from 5-9. This extract will also contain a small amount (6-10%) of free sugars (the monosaccharides fructose and glucose and the disaccharide sucrose), present in the starting root material and not a result of hot water extraction.[7,9]

Inulin can be further processed into more purified inulin-type prebiotic products (oligofructose or inulin HP). Total enzymatic hydrolysis results in monosaccharide molecules of fructose and glucose. Partial enzymatic hydrolysis can produce oligofructose mixtures with a DP ranging from a hot-water extracted inulin to a pure mix of completely enzymatically hydrolyzed monosaccharides. An endoinulase is used for partial enzymatic hydrolysis of inulin.[7,9,24]

Depending on the DPmax, DPav, DP range, and the amount of free sugars desired, the degree of partial hydrolysis can produce oligofructose products with different portfolios of fructans and free sugars. To appropriately compare products, the DPmax, DPav, DP range, and free-sugar content must be known.

Whether or not inulin undergoes partial enzymatic hydrolysis to produce oligofructose, physical separation techniques can be applied to produce products of higher purity and more uniformity. Using partial hydrolysis and/or physical separation techniques, products with 99-percent purity can be produced.[7,9]

Figure 3. *Cichorium intybus* (Chicory)

Because of additional processing, commercially available products with the generic names inulin or oligofructose are not identical in purity. Standard inulin (92% fructans and 8-10% free sugars) and low-sugar versions of inulin (99.5% fructans and ~0.5% sugars) are both referred to as inulin. Oligofructose syrups with ~60-percent fructans and ~40-percent sugars are commercially available as are syrups with ~95-percent fructans and ~5-percent sugars. Oligofructose syrups and powders with other specifications are also commercially available.[9]

Production of FOS

FOS is produced by an entirely different method. Using the fungal enzyme beta-fructosidase, derived from *Aspergillus niger*, FOS is enzymatically synthesized using a process called transfructosylation. The starting molecule used is sucrose, and the enzyme activity sequentially adds fructose units with new beta (2–1) linkages placed in the chain. Unlike inulin and oligofructose, which have all glycosidic bonds between fructose units in the beta (2–1) configuration, transfructosylation does not result exclusively in beta (2–1) fructosyl-fructose glycosidic bonds; other linkages occur in limited numbers.[7-9,24]

Enzymatic synthesis from glucose produces short-chain, low-molecular weight inulin-type fructans with a DP range of 2-4.[8,9,24]

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Short-chain fructan-type prebiotics produced in this manner typically have a DPmax of 4, DPav of approximately 3.6, and DP range from 2-4. Because the starting material is a sucrose molecule, the fructans in these mixes are of the GF_n type. As such, FOS has higher proportions of glucose units in the finished product than oligofructose, inulin, or inulin HP.[8]

Similar to inulin and oligofructose, FOS products vary in their free-sugar content. During enzymatic synthesis, some glucose and fructose molecules are formed as by-products. FOS can also contain unreacted sucrose. These free sugars can be removed or left in the finished product depending on the sweetness characteristics desired.[24]

Food Applications and Product Labeling

Inulin-type prebiotics are increasingly being used for food applications. This has potential clinical relevance since consumers might be consuming sufficient quantities of inulin-type prebiotics in foods and beverages to generate physiological responses, including gastrointestinal side effects.

Because inulin, oligofructose, and FOS are classified as soluble fibers they can be used as a means of increasing dietary fiber or to replace sugars or fats. Depending on the taste, texture, and other attributes desired, different mixtures are considered for inclusion in food products. In these applications they are considered to be a functional food ingredient, added to make health claims and/or persuade the consumer the product is a healthier choice than one that does not contain inulin-type prebiotics.[9]

Inulin-type prebiotics have a wide range of food applications, although they are not suitable for use in soft drinks and fruit jams because the acids in these foods hydrolyze the inulin-type fructans into monosaccharides.[9]

Fiber Applications

Inulin, oligofructose, and FOS are soluble fibers. According to Niness, "(Unlike other fibers) they have no off flavors and may be used to add fiber without contributing viscosity. These properties allow the formulation of high fiber foods that look and taste like standard food formulations. It is an invisible way to add fiber to foods."[24] The fiber claim on a nutritional label, without the taste and texture issues of most dietary fibers, is part of the reason for the growing use of inulin-type prebiotics in food applications.

Applications as a Fat Replacer

Certain mixes of inulin-type prebiotics can act as potential fat replacers in foods. Using a specific processing technique inulin is combined with water to produce the same texture and mouth feel as fat. As a fat replacement, this patented inulin-type prebiotic can be used in water-based foods such as dairy products and spreads, but not dry foods.[9]

Long-chain, high-molecular weight inulin HP is most desirable as a fat replacer. Longer chain lengths reduce the solubility of inulin-type fructans and result in the formation of what Niness describes as "inulin microcrystals" when mixed with water or milk, which are not discretely perceptible and have a smooth, creamy mouth feel. According to Niness, inulin HP has "almost twice the fat mimetic characteristics of standard inulin, with no sweetness contribution." When inulin-type prebiotics are used to produce low-fat spreads, for example, inulin HP is the preferred choice because of its superior fat mimetic properties and lack of sweetness.[24]

Application as a Sugar Replacer

Certain inulin-type prebiotics have properties that make them suitable as sugar replacers. In contrast to longer-chain, higher-molecular weight inulin-type fructans that are less soluble and fat mimetic, shorter-chain, lower-molecular weight oligofructose and FOS are preferred as sugar replacers. These shorter-chain oligomers are more soluble than sucrose, possess functional qualities similar to sugar or glucose syrup, and can have ~30-50 percent the sweetness of table sugar.[24]

As noted previously, inulin produced by hot-water extraction of chicory root produces ~8- to 10-percent free sugars, but this inulin only has a slight sweet taste.[7,9] Further processing techniques must be applied to produce something sweet enough to be a suitable sugar replacer. When starting from inulin, enzymatic hydrolysis can produce an oligofructose syrup or powder with more free sugars. A sweeter inulin-type fructan mix can also be produced from the synthesis of FOS from sucrose – a process that produces relatively high amounts of free sugars.[7,9]

Oligofructose or FOS syrups or powders typically do not have sufficient sweetness compared to sugar – ~30-50 percent the sweetness of sugar and are often combined with an intense sweetener to obtain a desired degree of sweetness. According to Coussement,

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[one of] "…the most common uses of FOS in foods is in combination with artificial sweeteners, since it acts in combination with them to improve their sweetness profiles while eliminating some of their aftertaste."[9] Niness reiterates this point, stating that, "Oligofructose is often used in combination with high intensity sweeteners to replace sugar, provide a well-balanced sweetness profile and mask the aftertaste of aspartame or acesulfame-k."[24]

In instances where a product containing oligofructose or FOS is being used as a sugar replacer, the objective is to convince consumers they are consuming something different than sugar – the inulin-type prebiotic – when in fact the sweet taste will be coming from free sugars. Food manufacturers can legally tout the presumed health benefits attributed to prebiotics because there is no labeling requirement to differentiate on food labels whether the oligofructose or FOS is low or high in free sugars.

Labeling of Inulin-type Prebiotics

Just as there is ambiguity in how the terms inulin, oligofructose, and FOS are used in research, there is ambiguity in how these terms appear on food labels. The hot-water extract of chicory is referred to as inulin, no matter how much free sugar it contains.[9] The terms FOS and oligofructose are used interchangeably and, like inulin, need not disclose the amount of free sugar.[9]

Inulin, oligofructose, and FOS contain some percent of fructose and glucose monosaccharides and sucrose disaccharides, unless additional processing is conducted to remove them. According to Coussement, it is legally permissible to not list these sugars on a food label since they are not added to the inulin-type prebiotic.[9]

The inulin produced from hot-water extraction of chicory root contains 8- to 10-percent free sugars. Oligofructose produced by partial hydrolysis of inulin or FOS synthesized from sucrose both contain higher amounts of naturally occurring free sugars, unless they are removed. Since these free sugars are considered to be a constituent of the inulin, oligofructose, or FOS, the accepted convention is they do not need to be listed as additional ingredients, although sugar content for a total product must be listed in the "Nutrition Facts" portion of a food label.[9]

In many countries, including the United States, the fructan molecules in inulin, oligofructose, and FOS are considered fiber from a food-labeling perspective, whether the fructan has a DP of 2 or 60. They are by convention listed as "soluble dietary fiber" under the nutrition facts.[9]

Several studies have attempted to determine the caloric value of FOS in human subjects. Using different methodologies, results have ranged from 1.0-2.8 kcal/g.[25-28] While it has not been investigated, it is possible that FOS (and inulin-type prebiotics in general) with differing proportions of free sugars might have different caloric values. Although no studies have confirmed the caloric value of inulin or oligosaccharides, some authors believe because they are structurally similar to FOS they have similar caloric values.[27] Independent of the caloric value of a specific inulin-type prebiotic, this scientific value can be in conflict with legal labeling requirements. For example, in Europe soluble fiber is considered to have "0" kcal/g, while in the United States it is considered to have 4 kcal/g.[9]

Metabolic Fate of Inulin-type Prebiotics

Inulin, oligofructose, and FOS resist enzymatic digestion in the upper gastrointestinal tract, reaching the colon virtually intact where they undergo bacterial fermentation.[7] Although inulin-type prebiotics induce growth of Bifidobacteria, they do not exert their effects in the same portion of the large bowel,[7] as DP influences where in the colon fermentation occurs.

Inulin, oligofructose, and FOS are fully metabolized by the colonic microflora. The end products of fermentation are gases (such as carbon dioxide and hydrogen), lactate, and short-chain fatty acids (including acetate, propionate, and butyrate). Increased hydrogen concentrations can be observed by breath hydrogen testing.[29-31] Colonic bacterial fermentation of inulin-type prebiotics, and the by-products produced, acidify the colonic content, increase bacterial biomass (and consequently fecal mass), and modify the composition of the microflora. The primary stimulating effect of prebiotics on gut ecology is stimulation of Bifidobacteria species growth.[2]

The DP appears to exert some influence on the metabolic response to specific inulin-type prebiotic compounds – including potential side effects. For example, fermentation of FOS appears to occur primarily in the proximal colon;[29,31,32] whereas, a higher proportion of the fructans in inulin appear to survive transit through the proximal colon. As a result, inulin might

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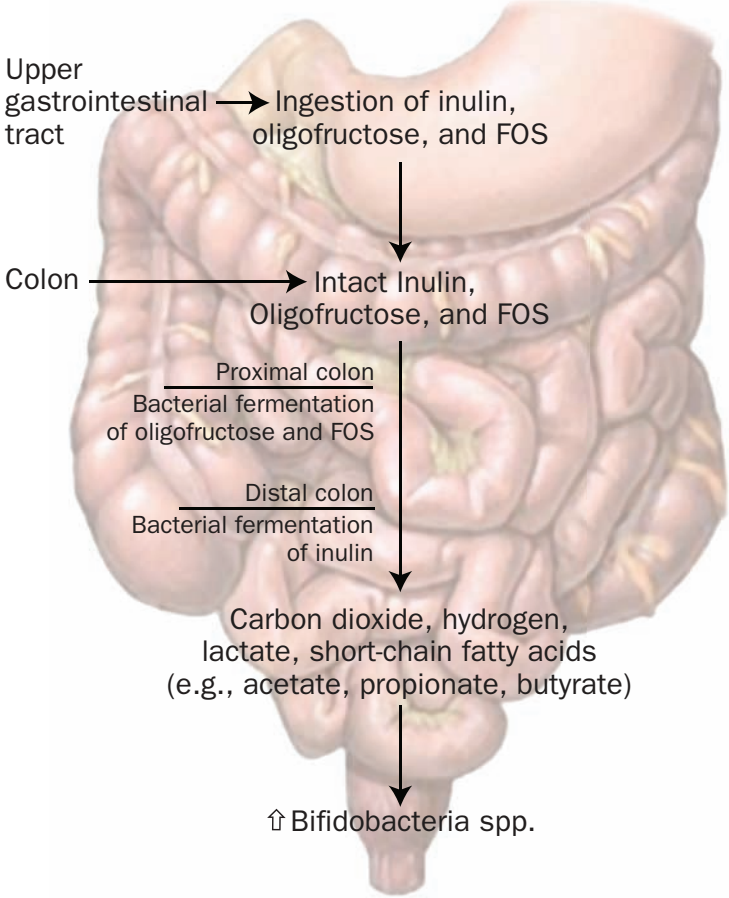
Figure 4. Metabolic Fate of Inulin-type Prebiotics



potentially have more positive effects on distal colonic fermentation and bacterial populations than a shorter-chain fructan-type prebiotic like FOS or oligofructose, which would be more metabolically active in the proximal colon (Figure 4).

Possibly as a result of the relative resistance to enzymatic degradation in the proximal colon, the transit time of fructans with greater DP is reportedly longer than that of short-chain fructans. Rumessen and Gudmand-Hoyer used a single-blind, crossover, randomized trial with 10 healthy adults to test the difference in intestinal transit times between oligofructose and inulin. Orocecal transit times were calculated using changes in breath hydrogen profiles. The estimated average transit time for inulin (51% of fructans with DP >12) was 75 minutes compared to 30 minutes for oligofructose (100% of fructans with DP <10). The researchers also concluded that shorter chain length (smaller DP) results in increased abdominal side effects.[31] Regarding inulin HP, theoretically more of the fructans reach the distal colon compared to inulin. Because the biological activity of inulin-type prebiotics appears to depend partially on the molecular size, it is particularly important to consider the molecular size distribution when reviewing clinical research.

Inulin-type Prebiotics and Growth of Specific Gut Microorganisms

Overview of Prebiotic Activity

The human gut harbors a complex community of bacteria. Part of the goal of prebiotic supplementation is to alter this community and, consistent with Roberfroid's updated definition, to exert positive changes "in the composition and/or activity in the gastrointestinal microflora…."[2] A number of studies have investigated the ability of inulin-type prebiotics to accomplish this goal. Current consensus is that inulin, oligofructose, and FOS are bifidogenic in infants and adults, although there appears to be significant inter-individual differences in bifidogenic response. In addition to having the potential to positively influence gut microflora, research has addressed the concern that inulin-type prebiotics might promote the growth of undesirable gut microorganisms.

In general, studies investigating the impact of inulin-type prebiotics on human gut flora have reported they promote the growth of Bifidobacteria, although the studies have not been consistent, and effects on other gut organisms have also been inconsistent.[11,13,18,33-47]

Some of the inconsistency might be due to the use of different inulin-type prebiotics – FOS, oligofructose, inulin, inulin HP. In addition, inconsistency arises because individuals do not have identical gut flora responses to inulin-type prebiotics. Both issues will be discussed in more detail.

According to Roberfroid two questions regarding prebiotic supplementation are regularly posed:[2] are different inulin-type prebiotics equally effective and can

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a dose-effect be established? These questions have yet to be definitely answered.

In terms of addressing the first question, while independent studies have been conducted on FOS, oligofructose, inulin, inulin HP, oligofructose-enriched inulin, and oligofructose-enriched inulin HP for their ability to influence gut flora, no comparative studies have been conducted. In the studies that investigated individual inulin-type prebiotics, the dose, study population, length of supplementation, and time intervals for microbiological analysis have varied. So, not surprisingly, reported results are not consistent. Despite these limitations and the need for more comparative research, Roberfroid reports that the prebiotic activity of different inulin-type prebiotics appear to be similar.[2]

Prebiotic Activity of FOS

Studies of FOS consistently report bifidogenic activity. Reported effects on other organisms are inconsistent, with some reporting no change, others a decrease, and others an increase in anaerobes. In the one study that continued to analyze stool samples following discontinuation of FOS, the potential for a rebound increase in undesirable organisms after cessation of FOS was suggested.[13]

Buddington et al gave 4 g FOS daily to 12 healthy subjects between days 7 and 32 of a 42-day controlled diet period. FOS supplementation increased total anaerobes and Bifidobacteria as assessed by stool culture. The species of Enterobacteriaceae and Bifidobacteria were not distinguished.[37]

Gibson et al reported that FOS increased the proportion of Bifidobacteria from 6-22 percent (assessed by stool cultures) in healthy adults during two weeks of supplementing 15 g daily in three divided doses. Significant decreases in Bacteroides, Clostridia, and Fusobacteria were also reported after two weeks of supplementation.[38]

Bouhnik et al gave FOS or placebo to 20 healthy volunteers who were observed for three consecutive 12-day periods (baseline, supplementation, and follow-up). During the 12-day ingestion period, subjects received 12.5 g/day FOS or sucrose as the placebo in three divided doses. FOS ingestion increased fecal Bifidobacteria counts and had no significant effect on fecal total anaerobes as assessed by stool culture.[33]

Bouhnik et al also conducted a longer-term study of FOS in 12 elderly volunteers (mean age 69) maintained on a controlled diet and given 8 g FOS daily in two divided doses. After four weeks, fecal Bifidobacteria counts were significantly increased compared to the pre-intervention observation period as assessed by stool culture. Four weeks following discontinuation of FOS supplementation, fecal Bifidobacteria counts returned to approximately initial levels, indicating supplementation had no lasting bifidogenic effect. Total anaerobe counts did not change with FOS supplementation, but were statistically decreased following discontinuation of FOS. No significant differences in fecal Clostridium counts were observed during FOS supplementation; however, a statistically significant rebound increase was observed during the follow-up period compared to the basal and active FOS supplementation periods. No statistically significant differences in fecal Enterobacteriaceae counts were observed during FOS supplementation or after discontinuation.[13]

Prebiotic Activity of Oligofructose

Studies that supplemented oligofructose consistently report bifidogenic activity during the period of active supplementation; however, findings with other organisms such as Clostridia have been inconsistent.

In a double-blind study, Kapiki et al fed 56 preterm infants standard infant formulas with addition of either oligofructose or a placebo (maltodextrin) for 14 days within the first two weeks after birth. Oligofructose increased the average number of stool Bifidobacteria and the proportion of infants colonized with Bifidobacteria. Stool culture yielded a higher number of Bacteroides and a reduction in numbers of *Escherichia coli* and Enterococci in the oligofructose group.[47]

In a randomized, crossover study, Euler et al gave formula-fed, full-term infants (ages 2-6 weeks) a cow's milk formula with or without oligofructose; washout weeks preceded and followed a week of oligofructose-supplemented formula feeding. Culture-based microbial assessment was used. A statistically significant increase in Bifidobacteria was observed following seven days of oligofructose feeding; however, it was not maintained after discontinuation. No statistically significant changes in Lactobacilli occurred with oligofructose supplementation. *Clostridium difficile* counts increased

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during oligofructose supplementation and decreased toward baseline levels following discontinuation, while Enterococci counts increased significantly during oligofructose supplementation and remained significantly elevated one week following discontinuation.[11]

In a double-blind, placebo-controlled trial, Waligora-Dupriet et al supplemented the diet of 7- to 19-month-old healthy children with oligofructose for 21 days, then followed up 15 days after cessation of supplementation. A slight, but statistically significant, increase in Bifidobacteria was observed with oligofructose supplementation, along with a statistically significant decrease in Clostridia. Modifications in Bifidobacteria and Clostridia did not persist following cessation of oligofructose.[46]

Menne et al supplemented eight adult volunteers with 8 g oligofructose daily for five weeks. Culture-based microbial assessment was used. A statistically significant increase in Bifidobacteria was reported, while total anaerobes, Lactobacilli, Bacteroides, coliforms, and *Clostridia perfringens* were unchanged.[42]

Prebiotic Activity of Inulin and Inulin HP

Two studies, one of inulin and one of inulin HP, indicate a bifidogenic effect from both.

Kleessen et al provided inulin supplementation for 19 days to a group of 10 elderly women with constipation. The dose began at 20 g/day from days 1 to 8 and gradually increased to 40 g/day during days 9 to 11; this dose remained constant days 12 to 19. Inulin increased Bifidobacteria significantly and decreased Enterococci in number and Enterobacteriaceae in frequency, while no statistically significant changes in Clostridia, Bacteroides, or *Faecalibacterium prausnitzii* were observed.[40]

In a double-blind trial, Tuohy et al supplemented 10 healthy volunteers with 8 g inulin HP daily for 14 days. A statistically significant increase in Bifidobacteria was observed, in addition to a statistically significant increase in Clostridia count, although the magnitude of change in Clostridia was one-tenth that of Bifidobacteria. Ingestion of inulin HP did not have a statistically significant effect on total bacteria, Bacteroides, Lactobacilli, or Enterococci. The bifidogenic effect was most marked in volunteers with low starting levels of Bifidobacteria.[45]

Prebiotic Activity of Oligofructose-enriched Inulin or Oligofructose-enriched Inulin HP

Oligofructose-enriched inulin and oligofructose-enriched inulin HP have been investigated for effect on gut microflora. Gibson et al reported that daily substitution of 15 g sucrose with 15 g of an oligofructose-enriched inulin product increased levels of Bifidobacteria.[38]

Brunser et al conducted a randomized, double-blind, controlled clinical trial in which 113 young children (ages 1-2 years) received a milk formula with or without oligofructose-enriched inulin for three weeks following cessation of amoxicillin, and were compared to the period prior to the antibiotic. Amoxicillin decreased total fecal bacteria (by close to 30%) and increased *E. coli*. Fluorescence *in situ* hybridization was used as the microbial assessment method. Although counts of Bifidobacteria and Bacteroides were not altered by amoxicillin, they represented a higher proportion of the total bacterial population because of reduction in other types of fecal bacteria. Administration of oligofructose-enriched inulin significantly increased Bifidobacteria. Although an increase in Lactobacillus species was also noted, it did not reach statistical significance. Total fecal bacterial counts of *E. coli* rapidly returned to pre-antibiotic levels independent of prebiotic administration within one week of antibiotic cessation.[36]

Ramirez-Farias et al gave 12 volunteers 10 g oligofructose-enriched inulin HP daily for 16 days. Fluorescence *in situ* hybridization was used as the microbial assessment method. The proportion of Bifidobacteria was significantly increased; however, both the baseline abundance and the magnitude of the response to inulin were very different among individuals. Specific strains of Bifidobacteria were assessed prior to and following the intervention. Of all strains monitored, *B. adolescentis* demonstrated the greatest increase; *B. bifidum* increased significantly for the five volunteers for whom this species was present prior to active supplementation. With the exception of the gram-positive species *Faecalibacterium prausnitzii*, which increased from 10.3 percent of the total microbiota during the control period to 14.5 percent during supplementation, other bacterial groups remained unchanged.[48]

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Langlands et al gave an oligofructose-enriched inulin HP product (7.5 g oligofructose and 7.5 g inulin HP daily) for two weeks to 14 subjects recruited from colonoscopy waiting lists. Oligofructose-enriched inulin HP significantly increased mucosal Bifidobacteria and Lactobacilli in the proximal and distal colon as assessed by culture-based methods. No changes in total anaerobes, Clostridia, Bacteroides, or coliforms were reported.[41]

Dose-relationship to Prebiotic Activity

In terms of Roberfroid's second question – whether there is a dose-response effect – few studies have investigated this topic. Roberfroid tried to answer this question by reviewing individual trials to assess bifidogenic responses and doses of inulin-type prebiotics used. He concluded a limited dose effect exists, but went on to state, "…it is the fecal flora composition (especially the number of Bifidobacteria before the prebiotic treatment) characteristic of each individual that determines the efficacy of a prebiotic but not necessarily the dose itself."[2] So while some degree of dose-effect does exist, an individual's response is dictated by other factors in addition to dose.

Current evidence indicates modest daily intake of inulin-type prebiotics of 2.5-5 g can have a bifidogenic effect in adults,[35,38,43,49] and is possibly the low end of the therapeutic dosing range. The limited direct comparative data suggests a modest dose effect is observed for higher doses up to 10 g daily in adults.

Bouhnik et al conducted a dose-response study of FOS supplementation to 40 healthy volunteers. Subjects were randomly divided into five groups, with each group receiving a daily dose of 2.5, 5.0, 7.5, or 10 g FOS or placebo for seven days. Bifidobacteria counts increased in all FOS groups, with a positive correlation between the dose and fecal Bifidobacteria counts. Total anaerobes increased at the 10-g daily dose, but not at lower doses, while no significant differences were found for Bacteroides, Lactobacillus, or Enterobacteriaceae.[34] In an earlier study, Bouhnik et al also reported a dose-related bifidogenic response to FOS in healthy volunteers. Although doses of 10 g and 20 g daily resulted in statistically significant increases in fecal Bifidobacteria compared to lower doses, the authors concluded the optimal daily dose for increasing Bifidobacteria while minimizing side effects was 10 g.[34]

It is unclear whether there is a dose above which a modest dose-response effect ceases to exist. However, one study in infants suggests the possibility of a biphasic response. In the study cited earlier by Euler et al, formula-fed term infants were given a cow's milk formula with or without oligofructose at doses of 1.5 g oligofructose/liter or 3.0 g oligofructose/liter. While a bifidogenic effect was observed for both doses, the mean increase in Bifidobacteria was significantly greater with the lower dose.[10]

Taken as a whole, it appears some minimal dose of inulin-type prebiotic is needed to produce a bifidogenic effect. However, intraindividual response to an identical dose of the same inulin-type prebiotic, in terms of stimulation of total number of Bifidobacteria and individual Bifidobacteria species, can be extremely variable.

Influence of Existing Gut Flora on Prebiotic Activity

Several researchers believe an important parameter in terms of a bifidogenic effect (and possibly on growth stimulation of other microorganisms) might be presence and/or numbers of these organisms prior to supplementation.[50,51] Preliminary data suggests several possibilities. First, if an organism is present in low numbers it might be stimulated to grow to a greater extent than if it is present in higher numbers. Second, for at least some specific microorganisms, existence of the organism as a component of the individual's gut microflora might be a precondition to stimulating its growth.

Hidaka et al reported initial numbers of Bifidobacteria influence the prebiotic effect following oligofructose supplementation. They observed an inverse correlation between initial numbers and increase after supplementation.[51] Tuohy et al reported the bifidogenic effect of inulin HP was most marked in volunteers with low starting levels of Bifidobacteria.[45]

Ramirez-Farias et al also found variable bifidogenic responses. As discussed above, 10 g daily oligofructose-enriched inulin to 12 volunteers for 16 days resulted in significant intraindividual differences in both initial levels and specific strains of Bifidobacteria. They further reported *B. bifidum* only increased significantly for the five volunteers for whom this species was present prior to active supplementation.[48]

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In summary, growth of Bifidobacteria as well as other commensal or pathogenic intestinal microflora appear to depend, at least in part, on the presence of these bacteria in the intestinal tract prior to prebiotic supplementation.

Conclusions

Inulin-type prebiotics are not a single homogenous nutritional compound, but represent a category of products that have in common the presence of fructans of the inulin-type. They can differ in fructan chain lengths and the percent of free sugars. There is no uniform standard for naming different inulin-type prebiotics. The three generic terms encountered most frequently are inulin, oligofructose, and fructooligosaccharides/FOS. These terms are not used the same way in research articles. Other terms including scFOS, lcFOS, native inulin, and inulin HP are also used. It is currently not possible, based on name alone, to determine whether an inulin-type prebiotic product has been synthesized from sucrose or extracted from chicory root.

Inulin-type prebiotics are mixes of fructans of differing chain lengths (ranging from as few as two linked fructose molecules to as many as 60) and can contain varying amounts of free sugars (from 0-40%). The generic name listed as an ingredient on food labels and dietary supplement labels does not accurately convey which specific mixture the product contains.

When choosing between different inulin-type prebiotics based on label information, a consumer or physician does not have access to sufficient information to make an accurate comparison. For example, a consumer or physician wishing to avoid fructose is unable to determine whether FOS, oligofructose, or inulin contains free fructose. Similarly, there is no way to determine, based on ingredient name alone, whether inulin is the hot-water extract (with free sugars and fructans ranging from 2-60 DP) or a more purified product that has had the free sugars and oligosaccharides (DP <10) removed.

Based on existing evidence, inulin-type fructans with longer DP values are more resistant to bacterial fermentation in the proximal colon and more likely to be metabolically active in the distal colon. Conversely, inulin-type fructans with shorter DP values are more likely to be metabolized by bacteria in the proximal colon, with far fewer surviving transit to the distal colon. Functionally, this means more fructans from inulin should reach the distal colon than from FOS or oligofructose. The same is true for inulin HP compared to inulin; more fructans in inulin HP would reach the distal colon. Because some degree of the biological activity of inulin-type prebiotics might depend on molecular size, it is particularly important to consider the molecular size distribution when reviewing clinical research and in future studies designed to monitor therapeutic efficacy.

Side effects might also be influenced by DP length. Existing evidence suggests FOS and oligofructose are more likely than inulin to produce gastrointestinal side effects at similar doses, since their fructans are DP <10 (shorter chains). This also suggests inulin might produce slightly more gastrointestinal side effects than inulin HP, since there are no oligosaccharides in inulin HP.

Although inulin, oligofructose, and FOS are bifidogenic, the effects on other gut microorganisms as well as pathogenic organisms are inconsistent. There is also a great deal of intraindividual variability in bifidogenic and anaerobe responses to inulin-type prebiotics.

Some of this inconsistency might be due to testing methods used and could be eliminated as newer microbial testing methods are employed. Culture-based technologies, once widely used, were used in the majority of studies reviewed. Molecular assessment methods of bacterial identification include: (1) fluorescence *in situ* hybridization, (2) polymerase chain reaction, (3) direct community analysis, and (4) denaturing/temperature gradient gel electrophoresis. These are considered to be more reliable and can more specifically characterize the diversity of gut microflora.[2] Future use of molecular assessment methods may help to better characterize prebiotic activity as well as identify the possible presence of pathogenic organisms.

The minimum dose of inulin-type prebiotics to produce a bifidogenic effect appears to be at least 2.5 g daily. Current evidence suggests some degree of dose-response up to 10 g daily. Although it is not clear whether daily doses above 10 g promote greater bifidogenesis, they do appear to increase the incidence of side effects.

Several researchers believe a potentially more important factor influencing growth of Bifidobacteria, as well as other microorganisms, is the initial presence

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and counts of specific gut microorganisms prior to supplementation. While more research in this area is needed, it is at least possible that in order to boost the amounts of a specific Bifidobacteria species or other bacteria, that species must initially be present.

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